(54) Title: METHOD AND REAGENT FOR PREVENTION, INHIBITION OF PROGRESSION AND REGRESSION OF VASCULAR DISEASES

(57) Abstract

A nucleic acid molecule which blocks synthesis and/or expression of mRNAs associated with initial development, progression or regression of vascular disease. In particular are provided ribozyme sequences which cleave human or rabbit cholesterol ester transfer protein (CETP) mRNAs.

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## DESCRIPTION

### Method and Reagent for Prevention, Inhibition of Progression and Regression of Vascular Diseases

#### Background Of The Invention

This invention relates to the methods for the prevention, inhibition of progression and regression of vascular diseases, in particular, inhibition of
5 cholesterol ester transfer protein (CETP) inhibition.

The following is a discussion of relevant art, none of which is admitted to be prior art to the present invention.

Vascular diseases, which includes etiologies such as
10 peripheral vascular disease, coronary heart disease (CHD), stroke and restenosis, remain the leading cause of death and disability in the United States and throughout the world. In 1990 alone, approximately 500,000 people died in the United States from CHD. Although, diet and life
15 style can accelerate the rate of onset of vascular diseases, genetic predisposition leading to "dyslipidemia" is a major and significant factor attributing to vascular related deaths and disabilities (Anderson et al., 1987 JAMA 257, 2176). By "dyslipidemia" is meant abnormal
20 levels of lipoproteins in plasma.

A variety of risk factors have been identified that are associated with increased risk of vascular disease (Barr et al., 1951 Am. J. Med. 11, 480; Kannel et al., 1971 Ann. Intern. Med. 74, 1; Miller et al., 1975 Lancet
25 1, 16; Levy et al., 1984 Circulation 69, 325.; Lipid Research Clinics Program, 1984 JAMA 251, 351; Lipid Research Clinics Program, 1984 JAMA 251, 365; Anderson et al., 1987 JAMA 257, 2176; Blankenhorn et al., 1987 J. Am. Med. Assoc. 257, 3233.; Frick et al., 1987 N. Engl. J.
30 Med. 317, 1237; Expert Panel, 1988 Arch. Intern. Med. 148, 36.; Grundy et al., 1989 Arch. Intern. Med. 149, 505.; La Rosa, 1990 Am. J. Cardiol. 65, 7F-10F). Among these are

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the dyslipidemias of high levels of low density lipo-
proteins (LDL) and low levels of high density lipoproteins
(HDL), singly or in combination.  Often the ratio of HDL
cholesterol to that of LDL cholesterol is used to assess
5   risk of vascular disease.  Thus, a high ratio of HDL/LDL
cholesterol is desirable, and intervention to increase the
ratio by lowering LDL and elevating HDL, singly or in
combination is desirable.

       Familial   hypercholesterolemia   (FH),   a   genetic
10  disorder caused by defective or deficient LDL receptors
presents as a marked elevation in LDL and risk in vascular
disease (Goldstein et al., 1989 "Familial hypercholester-
olemia" In: The Metabolic Basis of Inherited Diseases, 6th
Ed., Schiver, C.R., Beaudet, A.L., Sly, W.S., and Valle,
15  D. editors, 1215).  Homozygous FH is a relatively rare
disorder (1 in 1,000,000).  Homozygous FH patients have
extremely high levels of LDL with very short life expect-
ancies.  Therapy for these individuals include liver
transplantation and LDL plasmaphoresis.  Heterozygous FH
20  is relatively common disorder (1 in 500).  Heterozygous FH
patients present with LDL levels approximately twice
normal and are at risk for developing premature athero-
sclerosis followed by the common sequelae associated with
vascular diseases, including myocardial infarction and
25  stroke.  Conventional therapy for heterozygous FH patients
generally includes HMG CoA reductase inhibitors alone or
in combination with bile acid sequestrants.  Human
prospective trials have demonstrated reduction in CHD
related endpoints in hypercholesterolemic subjects treated
30  with HMGCoA reductase inhibitors, bile acid sequestrants,
Nicotinic acid, and gemfibrozil (La Rosa, 1990 Am. J.
Cardiol. 65, 7F; Pedersen et al., 1994 Lancet 344, 1383).

       Other conditions, such as apoE 3/4 and apoE4/4 geno-
type are also associated with increased LDL elevation and
35  risk of CHD. However the direct causal relation between
apoE4 polymorphism, elevated LDL and increased risk is
unknown (Davignon et al., 1988 Arteriosclerosis 8, 1;

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Dallongeville et al., 1992 J. Lipid Res. 33, 447; Walden
et al., 1994 Ann. Intern. Med. 120, 1026).

Low levels of HDL or hypoalphalipoproteinemia is a
relatively common condition. The genetic basis for hypo-
5   alphalipoproteinemia is poorly understood, but likely
results from multiple factors related to genetic predis-
position and life style. Numerous prospective and retro-
spective studies have shown that HDL is inversely and
strongly correlated with vascular disease.   Therefore
10  treatment to elevate HDL levels is warranted (Grundy et
al., 1989 Arch. Intern. Med. 149, 505). It is also well
recognized, that plasma triglyceride elevation is gener-
ally associated with low HDL levels, and insulin resist-
ance (Reaven, 1988 Diabetes 37, 1595).   Conventional
15  therapies for elevated triglycerides and low HDL generally
include treatment with fibrates. Gemfibrozil, a compound
of this class, effectively lowers plasma triglycerides,
and moderately elevates HDL (Frick, 1987 N. Engl. J. Med.
317, 1237). In a large human prospective trial, gemfibro-
20  zil has been shown to cause a significant reduction in
vascular endpoints (Frick, 1987 supra).

The process termed reverse cholesterol transport
(RCT; Bailey, 1965 Exp. Cell. Res. 37, 175; Glomset, 1968
J. Lipid Res. 9, 155) is a mechanism resulting in a net
25  efflux of cholesterol present in peripheral tissues for
disposal in bile. This multistep "hypothesized" pathway
invokes removal of cholesteryl from peripheral tissues to
HDL.   Lecithin: cholesterol acyltransferase (LCAT), a
circulating plasma enzyme, primarily mediates the esteri-
30  fication of HDL cholesterol to cholesteryl esters. CETP,
also present in plasma, mediates HDL cholesteryl ester net
transfer to apolipoprotein B (apoB)-containing lipo-
proteins including very low density lipoproteins (VLDL),
intermediate density lipoproteins or remnants (IDL) and
35  LDL.   CETP-mediated cholesteryl ester enrichment of the
LDL precursors, VLDL and IDL, ultimately contributes to
the cholesterol content of LDL. Hepatic receptor-mediated

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LDL uptake, and a net flux of these delivered cholesteryl esters to the bile acid pool completes RCT. However, species that lack CETP (Oschry et al., 1982 *J. Lipid Res.* 23, 1099) or humans deficient in CETP (Koizumi et al.,

5    1985 *Atherosclerosis* 58, 175) are unable to effectively transfer cholesteryl esters formed in HDL to the LDL precursor pool. Although the break in this link of the RCT pathway might predictably result in a marked defi-ciency in peripheral tissue cholesterol egress,

10   surprisingly, this is not the case. In CETP deficient species, the HDL cholesteryl ester pool accumulates at the expense of the LDL pool. The HDL particles become enlarged and apoA-I, apoA-IV, and apoE-enriched (Brown et al., 1989 *Nature* 342, 448-451; Yamashita et al., 1990 *J.*

15   *Clin. Invest.* 86, 688; Bisgaier et al., 1991 *J. Lipid Res.* 32, 21). Particles containing apoE can effectively be delivered to the liver as whole particles by facilitated mechanisms, including those utilizing the LDL receptor and the LDL receptor related protein (LRP) (Goldstein et al.,

20   1985 *Ann. Rev. Cell Biol.* 1, 1; Mahley, 1988 *Science* 240, 622.; Beisiegel et al., 1989 *Nature* 341, 162; Bisgaier et al., 1989 *J. Biol. Chem.* 264, 862.). Thus, alternative mechanisms exist that facilitate tissue cholesterol egress and delivery of non-LDL cholesterol to liver in the

25   absence of CETP.

Thus, CETP inhibition may inhibit or eliminate the RCT pathway thereby preventing the reduction in size and density of HDL, prolonging HDL half-life, and resulting in increased HDL levels. Additionally, the lack of transport

30   of cholesteryl esters from HDL to apoB-containing lipo-proteins may reduce LDL concentrations. Both these effects would result in an elevation of the HDL to LDL ratio. As high HDL/LDL ratios and HDL levels have been associated with anti-atherogenicity, diminishing CETP

35   activity may prevent or inhibit progression and regression of vascular disease.

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CETP is a 74 kDa glycoprotein that facilitates neutral lipid (cholesteryl esters and triglycerides) transfer between plasma lipoproteins (Zilversmit et al., 1975 *Biochim. Biophys. Acta* 409, 393; Ha et al., 1982
5 *Comp. Biochem. Physiol.* 71B, 265; Drayna et al., 1987 *Nature* 327, 632; Hesler et al., 1987 *J. Biol. Chem.* 262, 2275; Swenson et al., 1987 *J. Biol. Chem.* 262, 16271; Hesler et al., 1988 *J. Biol. Chem.* 263, 5020; Nagashima et al., 1988 *J. Lipid Res.* 29, 1643; Pape et al., 1991
10 *Arterioscler. Thromb.* 11, 1759). In non-human primates and rabbits, hepatic non-parenchymal cells are likely the major synthetic source of CETP (Pape et al., 1991 *Arterioscler. Thromb.* 11, 1759; Pape et al., 1991 *J. Biol. Chem.* 266, 12829; Rea et al., 1993 *J. Lipid Res.* 34,
15 1901), in that these cells have the highest cellular content of CETP mRNA relative to total RNA. Abundant amounts of CETP mRNA has also been shown in hepatic parenchymal cells, adipose, and spleen and to a lesser extent in the intestine and heart.
20 The level of CETP activity between species is highly variable (Ha et al., 1982 *Comp. Biochem. Physiol.* 71B, 265; Bisgaier et al., 1993 *J. Lipid Res.* 34, 1625). In general, species with high CETP activity (*e.g.*, humans and rabbits) are susceptible to dietary induced atheroscler-
25 osis, while species with little or no CETP activity (*e.g.*, mice, rats and dogs) are resistant (Koizumi et al., 1985 *Atherosclerosis* 58, 175; Inazu et al., 1990 *N. Engl. J. Med.* 323, 1234; Agellon et al., 1991 *J. Biol. Chem.* 260, 10796; Bisgaier et al., 1991 *J. Lipid Res.* 32, 21; Marotti
30 et al., 1992 *Arterioscler. Thrombosis* 12, 736). Likewise, those species with little or no CETP activity have anti-atherosclerotic lipoprotein profiles: plasma HDL levels are elevated and LDL are reduced (Ha et al., 1982 *Comp. Biochem. Physiol.* 71B, 265). Infusions of inhibitory CETP
35 monoclonal or polyclonal antibodies into rabbits or infusion of CETP into rats will invert the lipoprotein profiles (Ha et al., 1985 *Biochim. Biophys. Acta.* 833,

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203; Abbey et al., 1989 <u>Biochim. Biophys. Acta</u> 1003, 20; Groener et al., 1989 <u>Biochim. Biophys. Acta</u> 1002, 93; Whitlock et al., 1989 <u>J. Clin. Invest.</u> 84, 129.). Unlike control mice of similar genetic background, CETP trans-
5    genic mice develop atherosclerotic lipoproteins and atherosclerosis (Marotti et al., 1992 <u>Arterioscler. Thromb.</u> 12, 736).

Recent studies of a Japanese family have shown that a deficiency in plasma CETP associated with marked eleva-
10   tion of HDL, its associated apolipoproteins (apoA-I, apoE, apoA-IV) and a rarity of coronary artery disease (Koizumi et al., 1985 <u>Atherosclerosis</u> 58, 175; Brown et al., 1989 <u>Nature</u> 342, 448; Inazu et al., 1990 <u>N. Engl. J. Med.</u> 323, 1234; Bisgaier et al., 1991 <u>J. Lipid Res.</u> 32, 21.; Ikewaki
15   et al., 1991 <u>Arterioscler. Thromb.</u> 11, 1400a; Koizumi et al., 1991 <u>Atherosclerosis</u> 90, 189). These individuals were identified through routine cholesterol screening and have no other hyperlipidemia related disease. The defect has been identified as a G to A substitution in the
20   fourteenth intron of CETP pre-messenger ribonucleic acid (RNA) (Brown et al., 1989 <u>Nature</u> 342, 448). This splice donor defect is also the cause of the deficiency in additional Japanese families (Inazu et al., 1990 <u>N. Engl. J. Med.</u> 323, 1234; Koizumi et al., 1991 <u>Atherosclerosis</u>
25   90, 189; Hirano et al., 1993 <u>Atherosclerosis</u> 100, 85). In a more recent study, the deficiency (both homozygous and heterozygous) has been shown to be associated with a large proportion of Japanese with hyperalphalipoproteinemia (Inazu et al., 1992 <u>Horm. Metab. Res.</u> 24, 284; Hirano et
30   al., 1993 <u>Atherosclerosis</u> 100, 85). A missense mutation at nucleotide 1506 (G for A) also has been identified in exon 15 of the CETP gene, resulting in a substation of a glycine for aspartic acid at amino acid 442 (Takahashi et al., 1993 <u>J. Clin. Invest.</u> 92, 2060). The two subjects
35   heterozygous for the missense mutation had three times the normal HDL levels. Overall these studies suggest that even partial reduction in CETP levels, as found in

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heterozygous individuals, is associated with elevated HDL. This apparently benign condition (CETP deficiency) has been coined the "longevity syndrome" (Koizumi et al., 1985 Atherosclerosis 58, 175).

5      Although CETP facilitates an equimolar exchange of neutral lipids, net transfer of cholesteryl ester to LDL occurs due to (1) concentration and core lipid composition of exchange partners and (2) residence time of lipoproteins (Nichols et al., 1965 J. Lipid. Res. 206; Pattnaik

10   et al., 1978 Biochim. Biophys. Acta 530, 428; Barter et al., 1979 Metabolism 28, 230). Under basal conditions (i.e., overnight fast), CETP facilitates transfer below maximal velocity, while postprandially CETP appears to facilitate transfer at or near maximal velocity (Tall et

15   al., 1986 J. Clin. Invest. 77, 1163; Mann et al., 1991 J. Clin. Invest. 88, 2059; Bisgaier et al., 1993 J. Lipid Res. 34, 1625). It is also likely, but has not been systematically shown, that individuals with elevated triglycerides would have elevated CETP activity (but not

20   necessarily increased CETP mass). In general, these subjects have reduced levels of HDL and elevated LDL. These consequences, in part may be the result of events facilitated by CETP.

The complete amino acid sequence of human, rabbit,
25   cynomolgus monkey and hamster CETP are known (Drayna et al., 1987 Nature 327, 632; Nagashima et al., 1988 J. Lipid Res. 29, 1643; Jiang et al., 1991 J. Biol. Chem. 266, 4631; Pape et al., 1991 Arterioscler. Thromb. 11, 1759). Human plasma levels are approximately 1-2 μg/ml, while

30   rabbit levels are approximately 4 μg/ml. Cholesterol feeding in rabbits elevates tissue CETP mRNA, plasma CETP, and maximal plasma activity approximately 4 fold (Quinet et al., 1990 J. Clin. Invest. 85, 357; McPherson et al., 1991 Arterioscler. Thromb. 11, 797). The protein is

35   stable to heat, limited proteolysis, but not oxidation. CETP has been mapped with neutral and inhibitory monoclonal antibodies and by site-directed mutagenesis

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(Hesler et al., 1987 J. Biol. Chem. 262, 2275; Hesler et
al., 1988 J. Biol. Chem. 263, 5020; Wang et al., 1991
Biochemistry 30, 3484). Stable transfection of the human
gene in CHO cells has been accomplished (Wang et al., 1991
5   Biochemistry 30, 3484; Wang et al., 1992 J. Biol. Chem.
267, 17487). However, the protein has not been crystal-
lized nor have the lipid binding domains been identified.
Furthermore, the mechanisms by which CETP facilitates
transfer are poorly understood.

10      Direct pharmacological inhibition of the existing
protein in plasma or targeting CETP gene expression might
lead to reduced plasma activity and result in a beneficial
lipoprotein profile (i.e., HDL elevation and LDL diminu-
tion) and a reduced risk of coronary heart disease.
15  However a synthetic compound approach for the direct
inhibition of the plasma CETP has not yet been promising
(Bisgaier et al., 1994 Lipids 29).

The gene encoding CETP is composed of 16 exons of
various sizes (32-250 bp) and spans approximately 25 kb on
20  the long arm (q12-21) of chromosome 16 (Lusis et al., 1987
Genomics 1, 232; Agellon et al., 1990 Biochemistry 29,
1372). Cloning and sequencing of the human CETP cDNA has
been reported and shown to contain an open reading frame
and 3' untranslated region of 1656 nucleotides in length
25  (Drayna et al., 1987 Nature 327, 632). Analysis of amino-
acid and nucleic acid sequence has indicated a protein
that is unique among eukaryotic species. A pentanucleo-
tide amino acid stretch in the precursor protein signal
peptide of CETP is conserved among the lipid metabolism
30  associated proteins, for example apoA-IV, apoA-I, and
lipoprotein lipase (Agellon et al., 1990 Biochemistry 29,
1372). This conservation occurs at both the nucleotide
and the amino-acid level. This small but highly conserved
region is found only in the precursor protein species and
35  is removed before secretion of the mature protein into the
blood stream. Other less conserved homologies have been
noted with two lipopolysaccaride binding proteins, bac-

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terial permeability increasing protein found in leukocyte granules and plasma lipopolysaccharide binding protein (Tall, 1993 J. Lipid Res. 34, 1255).

A single predominant splicing variant of the CETP
5   message has been identified and characterized. This variant CETP mRNA lacks exon 9 and accounts for between 14-46% of total CETP mRNA with the highest percentage of this variant seen in the spleen. While the function of this abundant splice variant is not clearly understood,
10  when coordinately expressed with full-length CETP in Chinese Hamster Ovary (CHO) cells, it was shown not to be secreted and capable of inhibiting secretion of the full length CETP protein (Quinet et al., 1993 J. Biol. Chem. 268, 16891).

15      A consequence of inhibiting CETP, besides that of favorably increasing the HDL/LDL cholesterol ratio, is a change in the distribution and level of apoE. In species lacking CETP (e.g., rats), during monoclonal antibody induced inhibition of CETP in hamsters, and in human CETP
20  deficiency, plasma apoE levels are elevated (Yamashita et al., 1990 J. Clin. Invest. 86, 688; Eto et al., 1990 Artery 17, 202; Hirano et al., 1993 Atherosclerosis 100, 85; Takahashi et al., 1993 J. Clin. Invest. 92, 2060; Bisgaier et al., 1991 J. Lipid Res. 32, 21; Evans et al.,
25  1994 J. Lipid Res. 35, 1634). Furthermore, HDL apoE-enrichment was observed (Evans et al., 1994 J. Lipid Res. 35, 1634). Recent in vitro studies have revealed mechanisms by which apoE-enriched HDL are protective (Yamada et al., 1992 J. Clin. Invest. 706; Saxena et al., 1993 J.
30  Biol. Chem. 268, 14812). Additional studies have also demonstrated that apoE deficiency causes profound and accelerated rates of atherosclerosis in mice, a species not normally susceptible to atherosclerosis (Plump et al., 1992 Cell 71, 343; Zhang et al., 1992 Science 258, 468).
35  Thus an expected and desirable consequence of CETP inhibition includes elevation of apoE-rich HDL. In apoE deficiency, overexpression of apoA-I can also afford protec-

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tion against atherosclerosis (Plump et al., 1994 *Proc.*
*Natl. Acad. Sci. USA* 91, 9607), and elevation of this
protein is also an expected consequence of CETP inhibition
(Koizumi et al., 1985 *Atherosclerosis* 58, 175; Eto et al.,
5    1990 *Artery* 17, 202; Hirano et al., 1993 *Atherosclerosis*
100, 85; Takahashi et al., 1993 *J. Clin. Invest.* 92,
2060).

There currently exists no practical therapeutic
treatment for interfering with or blocking CETP activity
10   in humans.  Although not practical, repetitive anti-CETP
combined with anti-LDL plasmaphoreseis resulted in favor-
able changes in LDL and HDL levels and HDL/LDL ratios in
a limited number of human studies (Davidson, U.S. Patent
5,279,540).   Although plasma CETP levels markedly
15   decreased with duration of plasmaphoresis treatments,
neither anti-CETP plasmaphoresis alone nor control non-
immune plasmaphoresis data were reported.  Several poten-
tial inhibitors are being explored in various laborator-
ies.  These inhibitors include monoclonal antibodies and
20   an inhibitor protein recently found in baboon plasma
tentatively identified as the N-terminal fragment of
apolipoprotein C-I (Kushwaha et al., 1993 *J. Lipid Res.*
1993, 1285; Kushwaha et al., WO 93/11782).  In the current
application, a ribozyme, antisense or 2-5A-antisense or
25   triplex DNA approach is described.  The advantage of these
approaches is their ability to selectively target specific
regions of the CETP mRNA.


Summary Of The Invention
The invention features novel nucleic acid-based
30   techniques [e.g., enzymatic RNA molecules (ribozymes),
antisense nucleic acids, 2-5A antisense chimeras, triplex
DNA] and methods for their use for: (1) treatment of
dyslipidemias by decreasing lipoprotein risk factors,
in particular, decreasing high levels of LDL or increasing
35   low levels of HDL, or a combination of both and (2) for
the prevention, inhibition of progression, and regression

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of vascular diseases, particularly, those diseases asso-
ciated with (but not limited to) peripheral vascular
disease, coronary heart disease, stroke, vascular compli-
cations of diabetes, transplant, atherectomy, and
5 angioplastic restenosis.

The uniqueness of the CETP coding region and protein
afford an increased safety margin when contemplating
sequence-specific therapeutics targeting of the mRNA, such
as ribozymes or antisense nucleic acids or 2-5A antisense
10 chimeras, since there would be a reduced likelihood of
non-specific activity from these therapeutics.

In a preferred embodiment, the invention features use
of nucleic acid-based techniques to treat lipoprotein risk
factors and/or prevent vascular diseases by inhibiting the
15 synthesis of cholestryl ester transfer protein (CETP).

Those in the art will recognize the other potential
targets, for e.g., apolipoprotein B, are also suitable for
treatment with nucleic acid-based techniques described in
the present invention.

20 By "inhibit" is meant that the activity of CETP or
level of mRNAs encoded by CETP is reduced below that
observed in the absence of the nucleic acid, particularly,
inhibition with ribozymes and preferably is below that
level observed in the presence of an inactive RNA molecule
25 able to bind to the same site on the mRNA, but unable to
cleave that RNA.

By "enzymatic nucleic acid (NA) molecule" it is meant
a nucleic acid molecule which has complementarity in a
substrate binding region to a specified gene target, and
30 also has an enzymatic activity which is active to specif-
ically cleave RNA in that target. That is, the enzymatic
nucleic acid molecule is able to intermolecularly cleave
RNA and thereby inactivate a target RNA molecule. This
complementarity functions to allow sufficient hybridiza-
35 tion of the enzymatic nucleic acid molecule to the target
RNA to allow the cleavage to occur. One hundred percent

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complementarity is preferred, but complementarity as low
as 50-75% may also be useful in this invention.

By "equivalent" RNA to CETP is meant to include those
naturally occurring RNA molecules associated with cardio-
5   vascular diseases in various animals, including human,
rabbit and monkey. Such a molecule will generally contain
some ribonucleotides, but the other nucleotides may be
substituted at the 2'-hydroxyl position and in other
locations with other moieties as discussed below.

10  By "antisense nucleic acid" is meant a non-enzymatic
nucleic acid molecule that binds to another RNA (target
RNA) by means of RNA-RNA or RNA-DNA or RNA-PNA (protein
nucleic acid; Egholm et al., 1993 Nature 365, 566) inter-
actions and alters the activity of the target RNA (for a
15  review see Stein and Cheng, 1993 Science 261, 1004).

By "2-5A antisense chimera" is meant, an antisense
oligonucleotide containing a 5' phosphorylated 2'-5'-
linked adenylate residues. These chimeras bind to target
RNA in a sequence-specific manner and activate a cellular
20  2-5A-dependent ribonuclease which in turn cleaves the
target RNA (Torrence et al., 1993 Proc. Natl. Acad. Sci.
USA 90, 1300).

By "triplex DNA" is meant an oligonucleotide that can
bind to a double-stranded DNA in a sequence-specific
25  manner to form a triple-strand helix. Triple-helix forma-
tion has been shown to inhibit transcription of the
targeted gene (Duval-Valentin et al., 1992 Proc. Natl.
Acad. Sci. USA 89, 504).

By "gene" is meant a nucleic acid that encodes an
30  RNA.

By "complementarity" is meant a nucleic acid that can
form hydrogen bond(s) with other RNA sequence by either
traditional Watson-Crick or other non-traditional types
(for example, Hoogsteen type) of base-paired interactions.

35  Six basic varieties of naturally-occurring enzymatic
RNAs are known presently. Each can catalyze the hydro-
lysis of RNA phosphodiester bonds in trans (and thus can

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cleave other RNA molecules) under physiological conditions. Table I summarizes some of the characteristics of these ribozymes. In general, enzymatic nucleic acids act by first binding to a target RNA. Such binding occurs

5    through the target binding portion of a enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base-

10   pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is

15   released from that RNA to search for another target and can repeatedly bind and cleave new targets.

The enzymatic nature of a ribozyme is advantageous over other technologies, since the concentration of ribozyme necessary to affect a therapeutic treatment is lower.

20   This advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly specific inhibitor, with the specificity of inhibition depending not only on the base-

25   pairing mechanism of binding to the target RNA, but also on the mechanism of target RNA cleavage. Single mismatches, or base-substitutions, near the site of cleavage can completely eliminate catalytic activity of a ribozyme.

Ribozymes that cleave the specified sites in CETP

30   mRNAs represent a novel therapeutic approach to vascular disease. Applicant indicates that ribozymes are able to inhibit the activity of CETP and that the catalytic activity of the ribozymes is required for their inhibitory effect. Those of ordinary skill in the art, will find

35   that it is clear from the examples described that other ribozymes that cleave these sites in CETP mRNAs may be readily designed and are within the invention.

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In preferred embodiments of this invention, the enzymatic nucleic acid molecule is formed in a hammerhead or hairpin motif, but may also be formed in the motif of a hepatitis delta virus, group I intron or RNaseP RNA (in

5    association with an RNA guide sequence) or _Neurospora_ VS RNA. Examples of such hammerhead motifs are described by Rossi et al., 1992, _Aids Research and Human Retroviruses_ 8, 183, of hairpin motifs by Hampel et al., EP0360257, Hampel and Tritz, 1989 _Biochemistry_ 28, 4929, and Hampel

10   et al., 1990 _Nucleic Acids Res._ 18, 299, and an example of the hepatitis delta virus motif is described by Perrotta and Been, 1992 _Biochemistry_ 31, 16; of the RNaseP motif by Guerrier-Takada et al., 1983 _Cell_ 35, 849, _Neurospora_ VS RNA ribozyme motif is described by Collins (Saville and

15   Collins, 1990 _Cell_ 61, 685-696; Saville and Collins, 1991 _Proc. Natl. Acad. Sci. USA_ 88, 8826-8830; Collins and Olive, 1993 _Biochemistry_ 32, 2795-2799) and of the Group I intron by Cech et al., U.S. Patent 4,987,071. These specific motifs are not limiting in the invention and

20   those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule of this invention is that it has a specific substrate binding site which is complementary to one or more of the target gene RNA regions, and that it have nucleotide sequences within

25   or surrounding that substrate binding site which impart an RNA cleaving activity to the molecule.

In a preferred embodiment the invention provides a method for producing a class of enzymatic cleaving agents which exhibit a high degree of specificity for the RNA of

30   a desired target. The enzymatic nucleic acid molecule is preferably targeted to a highly conserved sequence region of a target mRNAs encoding CETP proteins such that specific treatment of a disease or condition can be provided with either one or several enzymatic nucleic acids. Such

35   enzymatic nucleic acid molecules can be delivered exogenously to specific cells as required. Alternatively, the

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ribozymes can be expressed from DNA/RNA vectors that are delivered to specific cells.

Synthesis of nucleic acids greater than 100 nucleotides in length is difficult using automated methods, and
5 the therapeutic cost of such molecules is prohibitive. In this invention, small enzymatic nucleic acid motifs (e.g., of the hammerhead or the hairpin structure) are used for exogenous delivery. The simple structure of these molecules increases the ability of the enzymatic nucleic acid
10 to invade targeted regions of the mRNA structure. However, these catalytic RNA molecules can also be expressed within cells from eukaryotic promoters (e.g., Scanlon et al., 1991, Proc. Natl. Acad. Sci. USA, 88, 10591-5; Kashani-Sabet et al., 1992 Antisense Res. Dev.,
15 2, 3-15; Dropulic et al., 1992 J. Virol, 66, 1432-41; Weerasinghe et al., 1991 J. Virol, 65, 5531-4; Ojwang et al., 1992 Proc. Natl. Acad. Sci. USA 89, 10802-6; Chen et al., 1992 Nucleic Acids Res., 20, 4581-9; Sarver et al., 1990 Science 247, 1222-1225). Those skilled in the art
20 realize that any ribozyme can be expressed in eukaryotic cells from the appropriate DNA/RNA vector. The activity of such ribozymes can be augmented by their release from the primary transcript by a second ribozyme (Draper et al., PCT WO 93/23569, and Sullivan et al., PCT WO
25 94/02595, both hereby incorporated in their totality by reference herein; Ohkawa et al., 1992 Nucleic Acids Symp. Ser., 27, 15-6; Taira et al., 1991, Nucleic Acids Res., 19, 5125-30; Ventura et al., 1993 Nucleic Acids Res., 21, 3249-55; Chowrira et al., 1994 J. Biol. Chem. 269, 25856).
30 Such ribozymes are useful for the prevention of the diseases and conditions discussed above, and any other diseases or conditions that are related to the level of CETP activity in a cell or tissue. By "related" is meant that the inhibition of CETP mRNAs and thus reduction in
35 the level of protein activity will relieve to some extent the symptoms of the disease or condition.

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Ribozymes are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells. The nucleic acid or nucleic acid complexes can be locally administered to
5    relevant tissues ex vivo, or in vivo through injection, infusion pump or stent, with or without their incorporation in biopolymers. In preferred embodiments, the ribozymes have binding arms which are complementary to the sequences in Tables II, IV, VI and VII. Examples of such
10   ribozymes are shown in Tables III, V, VI and VII. Examples of such ribozymes consist essentially of sequences defined in these Tables. By "consists essentially of" is meant that the active ribozyme contains an enzymatic center equivalent to those in the examples, and
15   binding arms able to bind mRNA such that cleavage at the target site occurs. Other sequences may be present which do not interfere with such cleavage.

In another aspect of the invention, ribozymes that cleave target molecules and inhibit CETP activity are
20   expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors are preferably DNA plasmids or viral vectors. Ribozyme expressing viral vectors could be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alpha-
25   virus. Preferably, the recombinant vectors capable of expressing the ribozymes are delivered as described above, and persist in target cells. Alternatively, viral vectors may be used that provide for transient expression of ribozymes. Such vectors might be repeatedly administered as
30   necessary. Once expressed, the ribozymes cleave the target mRNA. Delivery of ribozyme expressing vectors could be systemic, such as by intravenous or intramuscular administration, by administration to target cells explanted from the patient followed by reintroduction into
35   the patient, or by any other means that would allow for introduction into the desired target cell.

17

By "vectors" is meant any nucleic acid- and/or viral-based technique used to deliver a desired nucleic acid.

In a preferred embodiment nucleic acids targeted to Exon 9 of CETP gene is described. A single predominant alternate-splicing variant of the CETP message that lacks exon 9 has been identified and characterized (Inazu et al., 1991 _Biochemistry_ 31, 2352; Quinet et al., 1993 _J. Biol. Chem._ 268, 16891). While the function of this abundant splice variant is not clearly understood, it is known to be not secreted and capable of inhibiting secretion of the full-length CETP protein. (Quinet et al. 1993 _J. Biol. Chem._ 16891). Inhibition of full-length CETP secretion is believed to occur due to a heterodimeric complex formation between the full-length and the spliced variant of CETP. This suggests that the spliced variant of CETP might be beneficial in regulating the plasma level of CETP. Nucleic acid-based therapeutics of this invention, therefore, may be selectively targeted to block the expression of exon 9-containing CETP.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.


Description Of The Preferred Embodiments

The drawings will first briefly be described.


Drawings

Figure 1 is a diagrammatic representation of the hammerhead ribozyme domain known in the art. Stem II can be ≥ 2 base-pair long.

Figure 2a is a diagrammatic representation of the hammerhead ribozyme domain known in the art; Figure 2b is a diagrammatic representation of the hammerhead ribozyme as divided by Uhlenbeck (1987, _Nature_, 327, 596-600) into a substrate and enzyme portion; Figure 2c is a similar diagram showing the hammerhead divided by Haseloff and Gerlach (1988, _Nature_, 334, 585-591) into two portions;

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and Figure 2d is a similar diagram showing the hammerhead divided by Jeffries and Symons (1989, <u>Nucl. Acids. Res.</u>, 17, 1371-1371) into two portions.

Figure 3 is a diagrammatic representation of the
5  general structure of a hairpin ribozyme. Helix 2 (H2) is provided with a least 4 base pairs (<u>i.e.</u>, n is 1, 2, 3 or 4) and helix 5 can be optionally provided of length 2 or more bases (preferably 3 - 20 bases, <u>i.e.</u>, m is from 1 - 20 or more). Helix 2 and helix 5 may be covalently linked
10  by one or more bases (<u>i.e.</u>, r is $\geq$ 1 base). Helix 1, 4 or 5 may also be extended by 2 or more base pairs (<u>e.g.</u>, 4 - 20 base pairs) to stabilize the ribozyme structure, and preferably is a protein binding site. In each instance, each N and N' independently is any normal or modified base
15  and each dash represents a potential base-pairing inter-action. These nucleotides may be modified at the sugar, base or phosphate. Complete base-pairing is not required in the helices, but is preferred. Helix 1 and 4 can be of any size (<u>i.e.</u>, o and p is each independently from 0 to
20  any number, <u>e.g.</u>, 20) as long as some base-pairing is maintained. Essential bases are shown as specific bases in the structure, but those in the art will recognize that one or more may be modified chemically (abasic, base, sugar and/or phosphate modifications) or replaced with
25  another base without significant effect. Helix 4 can be formed from two separate molecules, <u>i.e.</u>, without a connecting loop. The connecting loop when present may be a ribonucleotide with or without modifications to its base, sugar or phosphate. "q" is $\geq$ 2 bases. The connect-
30  ing loop can also be replaced with a non-nucleotide linker molecule. H, refers to bases A, U or C. Y refers to pyrimidine bases.

Figure 4 is a representation of the general structure of the hepatitis delta virus ribozyme domain known in the
35  art.

Figure 5 is a representation of the general structure of the self-cleaving VS RNA ribozyme domain.

19

Figure 6 is a schematic representation of an RNAseH accessibility assay. Specifically, the left side of Figure 6 is a diagram of complementary DNA oligonucleotides bound to accessible sites on the target RNA.

5  Complementary DNA oligonucleotides are represented by broad lines labeled A, B, and C. Target RNA is represented by the thin, twisted line. The right side of Figure 6 is a schematic of a gel separation of uncut target RNA from a cleaved target RNA. Detection of target

10 RNA is by autoradiography of body-labeled, T7 transcript. The bands common to each lane represent uncleaved target RNA; the bands unique to each lane represent the cleaved products.

## Ribozymes

15   Ribozymes of this invention block to some extent CETP production and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture and to cells or tissues in animal models of cardio-vascular disorders. Ribozyme cleavage of CETP encoded

20 mRNAs in these systems may alleviate disease symptoms.

## Target sites

Targets for useful ribozymes can be determined as disclosed in Draper et al., "Method and reagent for treatment of arthritic conditions U.S.S.N. 08/152,487,

25 filed 11/12/93, and hereby incorporated by reference herein in totality. Rather than repeat the guidance provided in those documents here, below are provided specific examples of such methods, not limiting to those in the art. Ribozymes to such targets are designed as

30 described in those applications and synthesized to be tested in vitro and in vivo, as also described.

The sequence of human and rabbit CETP mRNAs were screened for optimal ribozyme target sites using a computer folding algorithm. Hammerhead or hairpin

35 ribozyme cleavage sites were identified. These sites are

20

shown in Tables II, IV, VI and VII  (All sequences are 5'
to 3' in the tables) The nucleotide base position is noted
in the Tables as that site to be cleaved by the designated
type of ribozyme.  While rabbit and human sequences can be
5  screened and ribozymes thereafter designed, the human
targeted sequences are of most utility.   However, as
discussed in Stinchcomb et al., "Method and Composition
for Treatment of Restenosis and Cancer Using Ribozymes,"
filed May 18, 1994, U.S.S.N. 08/245,466, rabbit targeted
10  ribozymes may be useful to test efficacy of action of the
ribozyme prior to testing in humans.  The nucleotide base
position is noted in the Tables as that site to be cleaved
by the designated type of ribozyme.

Hammerhead or hairpin ribozymes are designed that
15  could bind and were individually analyzed by computer
folding (Jaeger et al., 1989 Proc. Natl. Acad. Sci. USA,
86, 7706) to assess whether the ribozyme sequences fold
into the appropriate secondary structure.  Those ribozymes
with unfavorable intramolecular interactions between the
20  binding arms and the catalytic core are eliminated from
consideration.  Varying binding arm lengths can be chosen
to optimize activity.  Generally, at least 5 bases on each
arm are able to bind to, or otherwise interact with, the
target RNA.

25      Referring to Figure 6, mRNA were screened for access-
ible cleavage sites by the method described generally in
McSwiggen, US Patent application 07/883,849 filed on May
1, 1992, entitled "Assay for ribozyme target site", hereby
incorporated by reference herein.  Briefly, DNA oligo-
30  nucleotides representing potential hammerhead or hairpin
ribozyme cleavage sites were synthesized.  A polymerase
chain reaction is used to generate substrates for T7 RNA
polymerase transcription from human and rabbit CETP cDNA
clones.  Labeled RNA transcripts are synthesized in vitro
35  from the templates. The oligonucleotides and the labeled
transcripts are annealed, RNAseH is added and the mixtures
are incubated for the designated times at 37°C.  Reactions

21

are stopped and RNA separated on sequencing polyacrylamide gels. The percentage of the substrate cleaved is determined by autoradiographic quantitation using a PhosphorImaging system. From these data, hammerhead or
5  hairpin ribozyme sites are chosen as the most accessible.

Ribozymes of the hammerhead or hairpin motif are designed to anneal to various sites in the mRNA message. The binding arms are complementary to the target site sequences described above. The ribozymes are chemically
10 synthesized. The method of synthesis used follows the procedure for normal RNA synthesis as described in Usman et al., 1987 J. Am. Chem. Soc., 109, 7845 and in Scaringe et al., 1990 Nucleic Acids Res., 18, 5433 and made use of common nucleic acid protecting and coupling groups, such
15 as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The average stepwise coupling yields are >98%. Inactive ribozymes are synthesized by substituting a U for $G_5$ and a U for $A_{14}$ (numbering from Hertel et al., 1992 Nucleic Acids Res., 20, 3252). Hairpin ribozymes are
20 synthesized in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 Nucl. Acids. Res., 20, 2835-2840). Ribozymes are also synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and Uhlenbeck, 1989, Methods Enzymol. 180, 51).
25 All ribozymes are modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-flouro, 2'-O-methyl, 2'-H (for a review see Usman and Cedergren, 1992 TIBS 17, 34). Ribozymes are purified by gel electrophoresis using
30 general methods or are purified by high pressure liquid chromatography (HPLC; See Usman et al., Synthesis, deprotection, analysis and purification of RNA and ribozymes, filed May, 18, 1994, U.S.S.N. 08/245,736 the totality of which is hereby incorporated herein by reference) and are
35 resuspended in water.

The sequences of the ribozymes that are chemically synthesized, useful in this study, are shown in Tables

22

III, V, VI and VII.  Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity.  For

5   example, stem-loop II sequence of hammerhead ribozymes listed in Tables III and V (5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion, and/or insertion) to contain any sequences provided a minimum of two base-paired stem structure can form.  Similarly, stem-loop IV

10  sequence of hairpin ribozymes listed in Tables VI and VII (5'-CACGUUGUG-3') can be altered (substitution, deletion, and/or insertion) to contain any sequence, provided a minimum of two base-paired stem structure can form.  The sequences listed in Tables III, V, VI and VII may be

15  formed of ribonucleotides or other nucleotides or non-nucleotides.  Such ribozymes are equivalent to the ribozymes described specifically in the Tables.


Optimizing Ribozyme Activity

        Ribozyme activity can be optimized as described by

20  Stinchcomb et al., supra.  The details will not be repeated here, but include altering the length of the ribozyme binding arms (stems I and III, see Figure 2c), or chemically synthesizing ribozymes with modifications that prevent their degradation by serum ribonucleases (see

25  e.g., Eckstein et al., International Publication No. WO 92/07065; Perrault et al., 1990 Nature 344, 565; Pieken et al., 1991 Science 253, 314; Usman and Cedergren, 1992 Trends in Biochem. Sci. 17, 334; Usman et al., International Publication No. WO 93/15187; and Rossi et

30  al., International Publication No. WO 91/03162, as well as Usman, N. et al. US Patent Application 07/829,729, and Sproat, European Patent Application 92110298.4 which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules,

35  modifications which enhance their efficacy in cells, and removal of stem II bases to shorten RNA synthesis times

23

and reduce chemical requirements. (All these publications
are hereby incorporated by reference herein.),

     Sullivan, et al., supra, describes the general
methods for delivery of enzymatic RNA molecules.
5    Ribozymes may be administered to cells by a variety of
methods known to those familiar to the art, including, but
not restricted to, encapsulation in liposomes, by ionto-
phoresis, or by incorporation into other vehicles, such as
hydrogels, cyclodextrins, biodegradable nanocapsules, and
10   bioadhesive microspheres. For some indications, ribozymes
may be directly delivered ex vivo to cells or tissues with
or without the aforementioned vehicles. Alternatively,
the RNA/vehicle combination is locally delivered by direct
injection or by use of a catheter, infusion pump or stent.
15   Other routes of delivery include, but are not limited to,
intravascular, intramuscular, subcutaneous or joint injec-
tion, aerosol inhalation, oral (tablet or pill form),
topical, systemic, ocular, intraperitoneal and/or intra-
thecal delivery. More detailed descriptions of ribozyme
20   delivery and administration are provided in Sullivan et
al., supra and Draper et al., supra which have been
incorporated by reference herein.

     Another means of accumulating high concentrations of
a ribozyme(s) within cells is to incorporate the ribozyme-
25   encoding sequences into a DNA or RNA expression vector.
Transcription of the ribozyme sequences are driven from a
promoter for eukaryotic RNA polymerase I (pol I), RNA
polymerase II (pol II), or RNA polymerase III (pol III).
Transcripts from pol II or pol III promoters will be
30   expressed at high levels in all cells; the levels of a
given pol II promoter in a given cell type will depend on
the nature of the gene regulatory sequences (enhancers,
silencers, etc.) present nearby. Prokaryotic RNA polymer-
ase promoters are also used, providing that the prokary-
35   otic RNA polymerase enzyme is expressed in the appropriate
cells (Elroy-Stein and Moss, 1990 Proc. Natl. Acad. Sci.
USA, 87, 6743-7; Gao and Huang 1993 Nucleic Acids Res.,

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21, 2867-72; Lieber et al., 1993 Methods Enzymol., 217, 47-66; Zhou et al., 1990 Mol. Cell. Biol., 10, 4529-37). Several investigators have demonstrated that ribozymes expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet et al., 1992 Antisense Res. Dev., 2, 3-15; Ojwang et al., 1992 Proc. Natl. Acad. Sci. USA, 89, 10802-6; Chen et al., 1992 Nucleic Acids Res., 20, 4581-9; Yu et al., 1993 Proc. Natl. Acad. Sci. USA, 90, 6340-4; L'Huillier et al., 1992 EMBO J. 11, 4411-8; Lisziewicz et al., 1993 Proc. Natl. Acad. Sci. USA, 90, 8000-4). The above ribozyme transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral or alphavirus vectors).

In a preferred embodiment of the invention, a transcription unit expressing a ribozyme that cleaves mRNAs encoded by CETP is inserted into a plasmid DNA vector or an adenovirus or adeno-associated virus DNA viral vector or a retroviral RNA vector. Viral vectors have been used to transfer genes and lead to either transient or long term gene expression (Zabner et al., 1993 Cell 75, 207; Carter, 1992 Curr. Opi. Biotech. 3, 533). The adenovirus vector is delivered as recombinant adenoviral particles. The DNA may be delivered alone or complexed with vehicles (as described for RNA above). The recombinant adenovirus or AAV particles are locally administered to the site of treatment, e.g., through incubation or inhalation in vivo or by direct application to cells or tissues ex vivo.

In another preferred embodiments, the ribozyme is administered to the site of CETP expression (e.g., liver cells) in an appropriate liposomal vesicle.

Example 1: CETP Hammerhead ribozymes

By engineering ribozyme motifs we have designed several ribozymes directed against CETP encoded mRNA

25

sequences.  These ribozymes are synthesized with modifica-
tions that improve their nuclease resistance.  The ability
of ribozymes to cleave target sequences in vitro was
evaluated.

5        Several common human cell lines, such as HepG2, are
available that can be induced to express endogenous CETP
for experimental purposes.  Alternatively, non-human cell
lines have been developed which constitutively express a
cDNA encoding for human CETP (Wang et al., 1991
10  Biochemistry 30, 3484; Wang et al., 1992 J. Biol. Chem.
267, 17487).  Additional lines expressing human or rabbit
full length or exon 9 deleted cDNA under the control of
inducible or constitutive promoters could readily be
developed by those skilled in the art.  Several rabbit
15  animal models of experimental hypercholesterolemia are
available.  New Zealand white rabbits fed with high
cholesterol diets have been shown to develop athero-
sclerotic disease (Clarkson et al., 1988 in Use of Animal
Models For Reasearch in Human Nutrition, Comparative
20  Animal Nutrition vol. 6, Bexnen and West, eds.) and
Watanabe rabbits are a model of homozygous FH (defective
LDL receptor) and present with increased cholesterol
levels and spontaneous development of atherosclerosis and
tendinous xanthomas (Watanbe, 1980 Atherosclerosis 36,
25  261).  CETP protein levels can be measured clinically or
experimentally by ELISA, or radioimmuno assay.  CETP
enzyme activity can be measured in vitro or ex vivo by the
use of a fluorescently labeled substrate (Bisgaier et al.,
1993 J. Lipid Res. 34, 1625; Bisgaier et al., 1994 Lipids
30  29, in press).  CETP encoded mRNA levels can be assessed
by Northern analysis, RNAse protection, primer extension
analysis or quantitative RT-PCR.  Ribozymes that block the
induction of CETP activity and/or CETP protein encoding
mRNAs by more than 20% in vitro can be identified.

35       RNA ribozymes and/or genes encoding them will be
delivered by either free delivery, liposome delivery,
cationic lipid delivery, adeno-associated virus vector

26

delivery, adenovirus vector delivery, retrovirus vector delivery or plasmid vector delivery in these animal model (e.g., transgenic mouse) experiments. One dose of a ribo- zyme vector that constitutively expresses the ribozyme or
5  one or more doses of a stable anti-CETP ribozyme or a transiently expressing ribozyme vector may reduce the incidence or severity of atherosclerotic lesions or heart disease.

Diagnostic uses

10     Ribozymes of this invention may be used as diagnostic tools to examine genetic drift and mutations within dis- eased cells or to detect the presence of CETP RNA in a cell.  The close relationship between ribozyme activity and the structure of the target RNA allows the detection
15 of mutations in any region of the molecule which alters the base-pairing and three-dimensional structure of the target RNA.  By using multiple ribozymes described in this invention, one may map nucleotide changes which are important to RNA structure and function in vitro, as well
20 as in cells and tissues.  Cleavage of target RNAs with ribozymes may be used to inhibit gene expression and define the role (essentially) of specified gene products in the progression of disease.  In this manner, other genetic targets may be defined as important mediators of
25 the disease.  These experiments will lead to better treatment of the disease progression by affording the possibility of combinational therapies (e.g., multiple ribozymes targeted to different genes, ribozymes coupled with known small molecule inhibitors, or intermittent
30 treatment with combinations of ribozymes and/or other chemical or biological molecules).  Other in vitro uses of ribozymes of this invention are well known in the art, and include detection of the presence of mRNAs associated with CETP_related condition.  Such RNA is detected by
35 determining the presence of a cleavage product after treatment with a ribozyme using standard methodology.

27

In a specific example, ribozymes which can cleave only wild-type or mutant forms of the target RNA are used for the assay. The first ribozyme is used to identify wild-type RNA present in the sample and the second ribo-
5  zyme will be used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA will be cleaved by both ribozymes to demonstrate the relative ribozyme efficiencies in the reactions and the absence of cleavage of the "non-
10 targeted" RNA species. The cleavage products from the synthetic substrates will also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus each analysis will require two ribozymes, two substrates and one unknown sample which
15 will be combined into six reactions. The presence of cleavage products will be determined using an RNAse pro-tection assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a polyacryl-amide gel. It is not absolutely required to quantify the
20 results to gain insight into the expression of mutant RNAs and putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (i.e., CETP) is adequate to establish risk. If probes of
25 comparable specific activity are used for both tran-scripts, then a qualitative comparison of RNA levels will be adequate and will decrease the cost of the initial diagnosis. Higher mutant form to wild-type ratios will be correlated with higher risk whether RNA levels are
30 compared qualitatively or quantitatively.
Other embodiments are within the following claims.

28

Table I
Characteristics of Ribozymes
Group I Introns
Size: ~200 to >1000 nucleotides.

5   Requires a U in the target sequence immediately 5' of the
    cleavage site.
    Binds 4-6 nucleotides at 5'side of cleavage site.
    Over 75 known members of this class. Found in Tetrahymena
    thermophila rRNA, fungal mitochondria, chloroplasts, phage

10  T4, blue-green algae, and others.
    RNAseP RNA (M1 RNA)
    Size: ~290 to 400 nucleotides.
    RNA portion of a ribonucleoprotein enzyme.  Cleaves tRNA
    precursors to form mature tRNA.

15  Roughly 10 known members of this group all are bacterial
    in origin.
    Hammerhead Ribozyme
    Size: ~13 to 40 nucleotides.
    Requires the target sequence UH immediately 5' of the

20  cleavage site.
    Binds a variable number nucleotides on both sides of the
    cleavage site.
    14 known members of this class.  Found in a number of
    plant pathogens (virusoids) that use RNA as the infectious

25  agent (Figure 1)
    Hairpin Ribozyme
    Size: ~50 nucleotides.
    Requires the target sequence GUC immediately 3' of the
    cleavage site.

30  Binds 4-6 nucleotides at 5'side of the cleavage site and
    a variable number to the 3'side of the cleavage site.
    Only 3 known member of this class.  Found in three plant
    pathogen (satellite RNAs of the tobacco ringspot virus,
    arabis mosaic virus and chicory yellow mottle virus) which

35  uses RNA as the infectious agent (Figure 3).
    Hepatitis Delta Virus (HDV) Ribozyme
    Size: 50 - 60 nucleotides (at present).

29

Cleavage of target RNAs recently demonstrated.

Sequence requirements not fully determined.

Binding sites and structural requirements not fully determined, although no sequences 5' of cleavage site are

5   required.

Only 1 known member of this class. Found in human HDV (Figure 4).

Neurospora VS RNA Ribozyme

Size: ~144 nucleotides (at present)

10  Cleavage of target RNAs recently demonstrated.

Sequence requirements not fully determined.

Binding sites and structural requirements not fully determined. Only 1 known member of this class. Found in Neurospora VS RNA (Figure 5).

15  Table II: Human CETP HH Target Sequence

| nt. Sequence | Target Sequence | nt. Sequence | Target Sequence |
|---|---|---|---|
| 9 | UGAAUCU C UGGGGCC | 440 | CUCCAUU C AGAACGU |
| 45 | AGAGCCU C AUGUUCC | 450 | AACGUGU C UGUGGUC |
| 50 | CUCAUGU U CCGUGGG | 457 | CUGUGGU C UUCAAGG |
| 51 | UCAUGUU C CGUGGGG | 459 | GUGGUCU U CAAGGGG |
| 72 | CGGACAU A CAUAUAC | 460 | UGGUCUU C AAGGGGA |
| 76 | CAUACAU A UACGGGC | 477 | CUGAAGU A UGGCUAC |
| 78 | UACAUAU A CGGGCUC | 483 | UAUGGCU A CACCACU |
| 85 | ACGGGCU C CAGGCUG | 506 | GCUGGGU A UUGAUCA |
| 100 | AACGGCU C GGGCCAC | 508 | UGGGUAU U GAUCAGU |
| 109 | GGCCACU U ACACACC | 512 | UAUUGAU C AGUCCAU |
| 110 | GCCACUU A CACACCA | 516 | GAUCAGU C CAUUGAC |
| 127 | GCCUGAU A ACCAUGC | 520 | AGUCCAU U GACUUCG |
| 148 | CCACAGU C CUGACCC | 525 | AUUGACU U CGAGAUC |
| 186 | GCCUGCU C CAAAGGC | 526 | UUGACUU C GAGAUCG |
| 198 | GGCACCU C GCACGAG | 532 | UCGAGAU C GACUCUG |
| 214 | CAGGCAU C GUGUGCC | 537 | AUCGACU C UGCCAUU |
| 226 | GCCGCAU C ACCAAGC | 544 | CUGCCAU U GACCUCC |
| 241 | CUGCCCU C CUGGUGU | 550 | UUGACCU C CAGAUCA |
| 249 | CUGGUGU U GAACCAC | 556 | UCCAGAU C AACACAC |

30

| | | | | | |
|---|---|---|---|---|---|
| | 274 | AGGUGAU C CAGACCG | 579 | UGUGACU C UGGUAGA | |
| | 285 | ACCGCCU U CCAGCGA | 584 | CUCUGGU A GAGUGCG | |
| | 286 | CCGCCUU C CAGCGAG | 612 | GACUGCU A CCUGUCU | |
| | 300 | GCCAGCU A CCCAGAU | 618 | UACCUGU C UUUCCAU | |
| 5 | 308 | CCCAGAU A UCACGGG | 620 | CCUGUCU U UCCAUAA | |
| | 310 | CAGAUAU C ACGGGCG | 621 | CUGUCUU U CCAUAAG | |
| | 334 | UGAUGCU C CUUGGCC | 622 | UGUCUUU C CAUAAGC | |
| | 337 | UGCUCCU U GGCCAAG | 626 | UUUCCAU A AGCUGCU | |
| | 346 | GCCAAGU C AAGUAUG | 634 | AGCUGCU C CUGCAUC | |
| 10 | 351 | GUCAAGU A UGGGUUG | 641 | CCUGCAU C UCCAAGG | |
| | 357 | UAUGGGU U GCACAAC | 643 | UGCAUCU C CAAGGGG | |
| | 367 | ACAACAU C CAGAUCA | 670 | GGUGGAU C AAGCAGC | |
| | 373 | UCCAGAU C AGCCACU | 681 | CAGCUGU U CACAAAU | |
| | 381 | AGCCACU U GUCCAUC | 682 | AGCUGUU C ACAAAUU | |
| 15 | 384 | CACUUGU C CAUCGCC | 689 | CACAAAU U UCAUCUC | |
| | 388 | UGUCCAU C GCCAGCA | 690 | ACAAAUU U CAUCUCC | |
| | 423 | GCCAAGU C CAUUGAU | 691 | CAAAUUU C AUCUCCU | |
| | 427 | AGUCCAU U GAUGUCU | 694 | AUUUCAU C UCCUUCA | |
| | 433 | UUGAUGU C UCCAUUC | 696 | UUCAUCU C CUUCACC | |
| 20 | 435 | GAUGUCU C CAUUCAG | 699 | AUCUCCU U CACCCUG | |
| | 439 | UCUCCAU U CAGAACG | 700 | UCUCCUU C ACCCUGA | |
| | 715 | AGCUGGU C CUGAAGG | 991 | GAGUCUU C CACUCGC | |
| | 730 | GACAGAU C UGCAAAG | 996 | UUCCACU C GCUGGCC | |
| | 742 | AAGAGAU C AACGUCA | 1009 | CCAAGGU A GCUUUCC | |
| 25 | 748 | UCAACGU C AUCUCUA | 1013 | GGUAGCU U UCCAGGA | |
| | 751 | ACGUCAU C UCUAACA | 1014 | GUAGCUU U CCAGGAU | |
| | 753 | GUCAUCU C UAACAUC | 1015 | UAGCUUU C CAGGAUG | |
| | 755 | CAUCUCU A ACAUCAU | 1030 | GCCGCCU C AUGCUCA | |
| | 760 | CUAACAU C AUGGCCG | 1036 | UCAUGCU C AGCCUGA | |
| 30 | 770 | GGCCGAU U UUGUCCA | 1056 | GACGAGU U CAAGGCA | |
| | 771 | GCCGAUU U UGUCCAG | 1057 | ACGAGUU C AAGGCAG | |
| | 772 | CCGAUUU U GUCCAGA | 1083 | UGGGGCU U CAACACC | |
| | 775 | AUUUUGU C CAGACAA | 1084 | GGGGCUU C AACACCA | |
| | 796 | CCAGCAU C CUUUCAG | 1102 | AGGAAAU C UUCCAAG | |
| 35 | 799 | GCAUCCU U UCAGAUG | 1104 | GAAAUCU U CCAAGAG | |
| | 800 | CAUCCUU U CAGAUGG | 1105 | AAAUCUU C CAAGAGG | |
| | 801 | AUCCUUU C AGAUGGA | 1114 | AAGAGGU U GUCGGCG | |

31

| | | | | | |
|---|---|---|---|---|---|
| | 814 | GAGACAU U GGGGUGG | 1117 | | AGGUUGU C GGCGGCU |
| | 826 | UGGACAU U UCCCUGA | 1125 | | GGCGGCU U CCCCAGC |
| | 827 | GGACAUU U CCCUGAC | 1126 | | GCGGCUU C CCCAGCC |
| | 828 | GACAUUU C CCUGACA | 1144 | | CCCAAGU C ACCGUCC |
| 5 | 842 | AGGUGAU C CCGUCAU | 1150 | | UCACCGU C CACUGCC |
| | 847 | AUCCCGU C AUCACAG | 1159 | | ACUGCCU C AAGAUGC |
| | 850 | CCGUCAU C ACAGCCU | 1174 | | CCAAGAU C UCCUGCC |
| | 858 | ACAGCCU C CUACCUG | 1176 | | AAGAUCU C CUGCCAA |
| | 861 | GCCUCCU A CCUGGAG | 1195 | | AGGGAGU C GUGGUCA |
| 10 | 870 | CUGGAGU C CCAUCAC | 1201 | | UCGUGGU C AAUUCUU |
| | 875 | GUCCCAU C ACAAGGG | 1205 | | GGUCAAU U CUUCAGU |
| | 884 | CAAGGGU C AUUUCAU | 1206 | | GUCAAUU C UUCAGUG |
| | 887 | GGGUCAU U UCAUCUA | 1208 | | CAAUUCU U CAGUGAU |
| | 888 | GGUCAUU U CAUCUAC | 1209 | | AAUUCUU C AGUGAUG |
| 15 | 889 | GUCAUUU C AUCUACA | 1224 | | GUGAAAU U CCUCUUU |
| | 892 | AUUUCAU C UACAAGA | 1225 | | UGAAAUU C CUCUUUC |
| | 894 | UUCAUCU A CAAGAAU | 1228 | | AAUUCCU C UUUCCAC |
| | 904 | AGAAUGU C UCAGAGG | 1230 | | UUCCUCU U UCCACGC |
| | 906 | AAUGUCU C AGAGGAC | 1231 | | UCCUCUU U CCACGCC |
| 20 | 916 | AGGACCU C CCCCUCC | 1232 | | CCUCUUU C CACGCCC |
| | 922 | UCCCCCU C CCCACCU | 1253 | | GCAACAU U CUGUAGC |
| | 930 | CCCACCU U CUCGCCC | 1254 | | CAACAUU C UGUAGCU |
| | 931 | CCACCUU C UCGCCCA | 1258 | | AUUCUGU A GCUUACA |
| | 933 | ACCUUCU C GCCCACA | 1262 | | UGUAGCU U ACACAUU |
| 25 | 954 | GGGGACU C CCGCAUG | 1263 | | GUAGCUU A CACAUUU |
| | 966 | AUGCUGU A CUUCUGG | 1269 | | UACACAU U UGAAGAG |
| | 969 | CUGUACU U CUGGUUC | 1270 | | ACACAUU U GAAGAGG |
| | 970 | UGUACUU C UGGUUCU | 1280 | | AGAGGAU A UCGUGAC |
| | 975 | UUCUGGU U CUCUGAG | 1282 | | AGGAUAU C GUGACUA |
| 30 | 976 | UCUGGUU C UCUGAGC | 1289 | | CGUGACU A CCGUCCA |
| | 978 | UGGUUCU C UGAGCGA | 1294 | | CUACCGU C CAGGCCU |
| | 988 | AGCGAGU C UUCCACU | 1302 | | CAGGCCU C CUAUUCU |
| | 990 | CGAGUCU U CCACUCG | 1305 | | GCCUCCU A UUCUAAG |
| | 1307 | CUCCUAU U CUAAGAA | 1569 | | UUUGGCU U CCCUGAG |
| 35 | 1308 | UCCUAUU C UAAGAAA | 1570 | | UUGGCUU C CUGAGC |
| | 1310 | CUAUUCU A AGAAAAA | 1592 | | GGUGGAU U UCCUCCA |
| | 1321 | AAAAGCU C UUCUUAA | 1593 | | GUGGAUU U CCUCCAG |

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| | 1323 | AAGCUCU U CUUAAGC | 1594 | UGGAUUU C CUCCAGA |
|---|---|---|---|---|
| | 1324 | AGCUCUU C UUAAGCC | 1597 | AUUUCCU C CAGAGCU |
| | 1326 | CUCUUCU U AAGCCUC | 1605 | CAGAGCU U GAGCUAG |
| | 1327 | UCUUCUU A AGCCUCU | 1611 | UUGAGCU A GAAGUCU |
| 5 | 1333 | UAAGCCU C UUGGAUU | 1617 | UAGAAGU C UCCAAGG |
| | 1335 | AGCCUCU U GGAUUUC | 1619 | GAAGUCU C CAAGGAG |
| | 1340 | CUUGGAU U UCCAGAU | 1629 | AGGAGGU C GGGAUGG |
| | 1341 | UUGGAUU U CCAGAUU | 1641 | UGGGGCU U GUAGCAG |
| | 1342 | UGGAUUU C CAGAUUA | 1644 | GGCUUGU A GCAGAAG |
| 10 | 1348 | UCCAGAU U ACACCAA | 1666 | CCAGGCU C ACAGCUG |
| | 1349 | CCAGAUU A CACCAAA | 1686 | CUGGUGU C UCCUCCA |
| | 1363 | AGACUGU U UCCAACU | 1688 | GGUGUCU C CUCCAGC |
| | 1364 | GACUGUU U CCAACUU | 1691 | GUCUCCU C CAGCGUG |
| | 1365 | ACUGUUU C CAACUUG | 1707 | UGGAAGU U GGGUUAG |
| 15 | 1371 | UCCAACU U GACUGAG | 1712 | GUUGGGU U AGGAGUA |
| | 1386 | AGCAGCU C CGAGUCC | 1713 | UUGGGUU A GGAGUAC |
| | 1392 | UCCGAGU C CAUCCAG | 1719 | UAGGAGU A CGGGAGAU |
| | 1396 | AGUCCAU C CAGAGCU | 1733 | UGGAGAU U GGCUCCC |
| | 1404 | CAGAGCU U CCUGCAG | 1738 | AUUGGCU C CCAACUC |
| 20 | 1405 | AGAGCUU C CUGCAGU | 1745 | CCCAACU C CUCCCUA |
| | 1413 | CUGCAGU C AAUGAUC | 1748 | AACUCCU C CCUAUCC |
| | 1420 | CAAUGAU C ACCGCUG | 1752 | CCUCCCU A UCCUAAA |
| | 1435 | UGGGCAU C CCUGAGG | 1754 | UCCCUAU C CUAAAGG |
| | 1444 | CUGAGGU C AUGUCUC | 1757 | CUAUCCU A AAGGCCC |
| 25 | 1449 | GUCAUGU C UCGGCUC | 1773 | CUGGCAU U AAAGUGC |
| | 1451 | CAUGUCU C GGCUCGA | 1774 | UGGCAUU A AAGUGCU |
| | 1462 | UCGAGGU A GUGUUUA | | |
| | 1467 | GUAGUGU U UACAGCC | | |
| | 1468 | UAGUGUU U ACAGCCC | | |
| 30 | 1469 | AGUGUUU A CAGCCCU | | |
| | 1477 | CAGCCCU C AUGAACA | | |
| | 1501 | UGAGCCU C UUCGACA | | |
| | 1503 | AGCCUCU U CGACAUC | | |
| | 1504 | GCCUCUU C GACAUCA | | |
| 35 | 1510 | UCGACAU C AUCAACC | | |
| | 1513 | ACAUCAU C AACCCUG | | |
| | 1525 | CUGAGAU U AUCACUC | | |

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|   | 1526 | UGAGAUU A UCACUCG |
|---|------|------------------|
|   | 1528 | AGAUUAU C ACUCGAG |
|   | 1532 | UAUCACU C GAGAUGG |
|   | 1542 | GAUGGCU U CCUGCUG |
| 5 | 1543 | AUGGCUU C CUGCUGC |
|   | 1563 | AUGGACU U UGGCUUC |
|   | 1564 | UGGACUU U GGCUUCC |


Table III:   Human CETP HH Ribozyme Sequence
nt.

| 10 | Position | HH Ribozyme Sequence |
|----|----------|----------------------|
|    | 9        | GGCCCCA CUGAUGAGGCCGAAAGGCCGAA AGAUUCA |
|    | 45       | GGAACAU CUGAUGAGGCCGAAAGGCCGAA AGGCUCU |
|    | 50       | CCCACGG CUGAUGAGGCCGAAAGGCCGAA ACAUGAG |
|    | 51       | CCCCACG CUGAUGAGGCCGAAAGGCCGAA AACAUGA |
| 15 | 72       | GUAUAUG CUGAUGAGGCCGAAAGGCCGAA AUGUCCG |
|    | 76       | GCCCGUA CUGAUGAGGCCGAAAGGCCGAA AUGUAUG |
|    | 78       | GAGCCCG CUGAUGAGGCCGAAAGGCCGAA AUAUGUA |
|    | 85       | CAGCCUG CUGAUGAGGCCGAAAGGCCGAA AGCCCGU |
|    | 100      | GUGGCCC CUGAUGAGGCCGAAAGGCCGAA AGCCGUU |
| 20 | 109      | GGUGUGU CUGAUGAGGCCGAAAGGCCGAA AGUGGCC |
|    | 110      | UGGUGUG CUGAUGAGGCCGAAAGGCCGAA AAGUGGC |
|    | 127      | GCAUGGU CUGAUGAGGCCGAAAGGCCGAA AUCAGGC |
|    | 148      | GGGUCAG CUGAUGAGGCCGAAAGGCCGAA ACUGUGG |
|    | 186      | GCCUUUG CUGAUGAGGCCGAAAGGCCGAA AGCAGGC |
| 25 | 198      | CUCGUGC CUGAUGAGGCCGAAAGGCCGAA AGGUGCC |
|    | 214      | GGCACAC CUGAUGAGGCCGAAAGGCCGAA AUGCCUG |
|    | 226      | GCUUGGU CUGAUGAGGCCGAAAGGCCGAA AUGCGGC |
|    | 241      | ACACCAG CUGAUGAGGCCGAAAGGCCGAA AGGGCAG |
|    | 249      | GUGGUUC CUGAUGAGGCCGAAAGGCCGAA ACACCAG |
| 30 | 274      | CGGUCUG CUGAUGAGGCCGAAAGGCCGAA AUCACCU |
|    | 285      | UCGCUGG CUGAUGAGGCCGAAAGGCCGAA AGGCGGU |
|    | 286      | CUCGCUG CUGAUGAGGCCGAAAGGCCGAA AAGGCGG |
|    | 300      | AUCUGGG CUGAUGAGGCCGAAAGGCCGAA AGCUGGC |
|    | 308      | CCCGUGA CUGAUGAGGCCGAAAGGCCGAA AUCUGGG |
| 35 | 310      | CGCCCGU CUGAUGAGGCCGAAAGGCCGAA AUAUCUG |
|    | 334      | GGCCAAG CUGAUGAGGCCGAAAGGCCGAA AGCAUCA |

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| | 337 | CUUGGCC CUGAUGAGGCCGAAAGGCCGAA AGGAGCA |
|---|---|---|
| | 346 | CAUACUU CUGAUGAGGCCGAAAGGCCGAA ACUUGGC |
| | 351 | CAACCCA CUGAUGAGGCCGAAAGGCCGAA ACUUGAC |
| | 357 | GUUGUGC CUGAUGAGGCCGAAAGGCCGAA ACCCAUA |
| 5 | 367 | UGAUCUG CUGAUGAGGCCGAAAGGCCGAA AUGUUGU |
| | 373 | AGUGGCU CUGAUGAGGCCGAAAGGCCGAA AUCUGGA |
| | 381 | GAUGGAC CUGAUGAGGCCGAAAGGCCGAA AGUGGCU |
| | 384 | GGCGAUG CUGAUGAGGCCGAAAGGCCGAA ACAAGUG |
| | 388 | UGCUGGC CUGAUGAGGCCGAAAGGCCGAA AUGGACA |
| 10 | 423 | AUCAAUG CUGAUGAGGCCGAAAGGCCGAA ACUUGGC |
| | 427 | AGACAUC CUGAUGAGGCCGAAAGGCCGAA AUGGACU |
| | 433 | GAAUGGA CUGAUGAGGCCGAAAGGCCGAA ACAUCAA |
| | 435 | CUGAAUG CUGAUGAGGCCGAAAGGCCGAA AGACAUC |
| | 439 | CGUUCUG CUGAUGAGGCCGAAAGGCCGAA AUGGAGA |
| 15 | 440 | ACGUUCU CUGAUGAGGCCGAAAGGCCGAA AAUGGAG |
| | 450 | GACCACA CUGAUGAGGCCGAAAGGCCGAA ACACGUU |
| | 457 | CCUUGAA CUGAUGAGGCCGAAAGGCCGAA ACCACAG |
| | 459 | CCCCUUG CUGAUGAGGCCGAAAGGCCGAA AGACCAC |
| | 460 | UCCCCUU CUGAUGAGGCCGAAAGGCCGAA AAGACCA |
| 20 | 477 | GUAGCCA CUGAUGAGGCCGAAAGGCCGAA ACUUCAG |
| | 483 | AGUGGUG CUGAUGAGGCCGAAAGGCCGAA AGCCAUA |
| | 506 | UGAUCAA CUGAUGAGGCCGAAAGGCCGAA ACCCAGC |
| | 508 | ACUGAUC CUGAUGAGGCCGAAAGGCCGAA AUACCCA |
| | 512 | AUGGACU CUGAUGAGGCCGAAAGGCCGAA AUCAAUA |
| 25 | 516 | GUCAAUG CUGAUGAGGCCGAAAGGCCGAA ACUGAUC |
| | 520 | CGAAGUC CUGAUGAGGCCGAAAGGCCGAA AUGGACU |
| | 525 | GAUCUCG CUGAUGAGGCCGAAAGGCCGAA AGUCAAU |
| | 526 | CGAUCUC CUGAUGAGGCCGAAAGGCCGAA AAGUCAA |
| | 532 | CAGAGUC CUGAUGAGGCCGAAAGGCCGAA AUCUCGA |
| 30 | 537 | AAUGGCA CUGAUGAGGCCGAAAGGCCGAA AGUCGAU |
| | 544 | GGAGGUC CUGAUGAGGCCGAAAGGCCGAA AUGGCAG |
| | 550 | UGAUCUG CUGAUGAGGCCGAAAGGCCGAA AGGUCAA |
| | 556 | GUGUGUU CUGAUGAGGCCGAAAGGCCGAA AUCUGGA |
| | 579 | UCUACCA CUGAUGAGGCCGAAAGGCCGAA AGUCACA |
| 35 | 584 | CGCACUC CUGAUGAGGCCGAAAGGCCGAA ACCAGAG |
| | 612 | AGACAGG CUGAUGAGGCCGAAAGGCCGAA AGCAGUC |
| | 618 | AUGGAAA CUGAUGAGGCCGAAAGGCCGAA ACAGGUA |

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      620   UUAUGGA  CUGAUGAGGCCGAAAGGCCGAA  AGACAGG
      621   CUUAUGG  CUGAUGAGGCCGAAAGGCCGAA  AAGACAG
      622   GCUUAUG  CUGAUGAGGCCGAAAGGCCGAA  AAAGACA
      626   AGCAGCU  CUGAUGAGGCCGAAAGGCCGAA  AUGGAAA
  5   634   GAUGCAG  CUGAUGAGGCCGAAAGGCCGAA  AGCAGCU
      641   CCUUGGA  CUGAUGAGGCCGAAAGGCCGAA  AUGCAGG
      643   CCCCUUG  CUGAUGAGGCCGAAAGGCCGAA  AGAUGCA
      670   GCUGCUU  CUGAUGAGGCCGAAAGGCCGAA  AUCCACC
      681   AUUUGUG  CUGAUGAGGCCGAAAGGCCGAA  ACAGCUG
 10   682   AAUUUGU  CUGAUGAGGCCGAAAGGCCGAA  AACAGCU
      689   GAGAUGA  CUGAUGAGGCCGAAAGGCCGAA  AUUUGUG
      690   GGAGAUG  CUGAUGAGGCCGAAAGGCCGAA  AAUUUGU
      691   AGGAGAU  CUGAUGAGGCCGAAAGGCCGAA  AAAUUUG
      694   UGAAGGA  CUGAUGAGGCCGAAAGGCCGAA  AUGAAAU
 15   696   GGUGAAG  CUGAUGAGGCCGAAAGGCCGAA  AGAUGAA
      699   CAGGGUG  CUGAUGAGGCCGAAAGGCCGAA  AGGAGAU
      700   UCAGGGU  CUGAUGAGGCCGAAAGGCCGAA  AAGGAGA
      715   CCUUCAG  CUGAUGAGGCCGAAAGGCCGAA  ACCAGCU
      730   CUUUGCA  CUGAUGAGGCCGAAAGGCCGAA  AUCUGUC
 20   742   UGACGUU  CUGAUGAGGCCGAAAGGCCGAA  AUCUCUU
      748   UAGAGAU  CUGAUGAGGCCGAAAGGCCGAA  ACGUUGA
      751   UGUUAGA  CUGAUGAGGCCGAAAGGCCGAA  AUGACGU
      753   GAUGUUA  CUGAUGAGGCCGAAAGGCCGAA  AGAUGAC
      755   AUGAUGU  CUGAUGAGGCCGAAAGGCCGAA  AGAGAUG
 25   760   CGGCCAU  CUGAUGAGGCCGAAAGGCCGAA  AUGUUAG
      770   UGGACAA  CUGAUGAGGCCGAAAGGCCGAA  AUCGGCC
      771   CUGGACA  CUGAUGAGGCCGAAAGGCCGAA  AAUCGGC
      772   UCUGGAC  CUGAUGAGGCCGAAAGGCCGAA  AAAUCGG
      775   UUGUCUG  CUGAUGAGGCCGAAAGGCCGAA  ACAAAAU
 30   796   CUGAAAG  CUGAUGAGGCCGAAAGGCCGAA  AUGCUGG
      799   CAUCUGA  CUGAUGAGGCCGAAAGGCCGAA  AGGAUGC
      800   CCAUCUG  CUGAUGAGGCCGAAAGGCCGAA  AAGGAUG
      801   UCCAUCU  CUGAUGAGGCCGAAAGGCCGAA  AAAGGAU
      814   CCACCCC  CUGAUGAGGCCGAAAGGCCGAA  AUGUCUC
 35   826   UCAGGGA  CUGAUGAGGCCGAAAGGCCGAA  AUGUCCA
      827   GUCAGGG  CUGAUGAGGCCGAAAGGCCGAA  AAUGUCC
      828   UGUCAGG  CUGAUGAGGCCGAAAGGCCGAA  AAAUGUC
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| | 842 | AUGACGG | CUGAUGAGGCCGAAAGGCCGAA | AUCACCU |
|---|---|---|---|---|
| | 847 | CUGUGAU | CUGAUGAGGCCGAAAGGCCGAA | ACGGGAU |
| | 850 | AGGCUGU | CUGAUGAGGCCGAAAGGCCGAA | AUGACGG |
| | 858 | CAGGUAG | CUGAUGAGGCCGAAAGGCCGAA | AGGCUGU |
| 5 | 861 | CUCCAGG | CUGAUGAGGCCGAAAGGCCGAA | AGGAGGC |
| | 870 | GUGAUGG | CUGAUGAGGCCGAAAGGCCGAA | ACUCCAG |
| | 875 | CCCUUGU | CUGAUGAGGCCGAAAGGCCGAA | AUGGGAC |
| | 884 | AUGAAAU | CUGAUGAGGCCGAAAGGCCGAA | ACCCUUG |
| | 887 | UAGAUGA | CUGAUGAGGCCGAAAGGCCGAA | AUGACCC |
| 10 | 888 | GUAGAUG | CUGAUGAGGCCGAAAGGCCGAA | AAUGACC |
| | 889 | UGUAGAU | CUGAUGAGGCCGAAAGGCCGAA | AAAUGAC |
| | 892 | UCUUGUA | CUGAUGAGGCCGAAAGGCCGAA | AUGAAAU |
| | 894 | AUUCUUG | CUGAUGAGGCCGAAAGGCCGAA | AGAUGAA |
| | 904 | CCUCUGA | CUGAUGAGGCCGAAAGGCCGAA | ACAUUCU |
| 15 | 906 | GUCCUCU | CUGAUGAGGCCGAAAGGCCGAA | AGACAUU |
| | 916 | GGAGGGG | CUGAUGAGGCCGAAAGGCCGAA | AGGUCCU |
| | 922 | AGGUGGG | CUGAUGAGGCCGAAAGGCCGAA | AGGGGGA |
| | 930 | GGGCGAG | CUGAUGAGGCCGAAAGGCCGAA | AGGUGGG |
| | 931 | UGGGCGA | CUGAUGAGGCCGAAAGGCCGAA | AAGGUGG |
| 20 | 933 | UGUGGGC | CUGAUGAGGCCGAAAGGCCGAA | AGAAGGU |
| | 954 | CAUGCGG | CUGAUGAGGCCGAAAGGCCGAA | AGUCCCC |
| | 966 | CCAGAAG | CUGAUGAGGCCGAAAGGCCGAA | ACAGCAU |
| | 969 | GAACCAG | CUGAUGAGGCCGAAAGGCCGAA | AGUACAG |
| | 970 | AGAACCA | CUGAUGAGGCCGAAAGGCCGAA | AAGUACA |
| 25 | 975 | CUCAGAG | CUGAUGAGGCCGAAAGGCCGAA | ACCAGAA |
| | 976 | GCUCAGA | CUGAUGAGGCCGAAAGGCCGAA | AACCAGA |
| | 978 | UCGCUCA | CUGAUGAGGCCGAAAGGCCGAA | AGAACCA |
| | 988 | AGUGGAA | CUGAUGAGGCCGAAAGGCCGAA | ACUCGCU |
| | 990 | CGAGUGG | CUGAUGAGGCCGAAAGGCCGAA | AGACUCG |
| 30 | 991 | GCGAGUG | CUGAUGAGGCCGAAAGGCCGAA | AAGACUC |
| | 996 | GGCCAGC | CUGAUGAGGCCGAAAGGCCGAA | AGUGGAA |
| | 1009 | GGAAAGC | CUGAUGAGGCCGAAAGGCCGAA | ACCUUGG |
| | 1013 | UCCUGGA | CUGAUGAGGCCGAAAGGCCGAA | AGCUACC |
| | 1014 | AUCCUGG | CUGAUGAGGCCGAAAGGCCGAA | AAGCUAC |
| 35 | 1015 | CAUCCUG | CUGAUGAGGCCGAAAGGCCGAA | AAAGCUA |
| | 1030 | UGAGCAU | CUGAUGAGGCCGAAAGGCCGAA | AGGCGGC |
| | 1036 | UCAGGCU | CUGAUGAGGCCGAAAGGCCGAA | AGCAUGA |

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     1056    UGCCUUG CUGAUGAGGCCGAAAGGCCGAA ACUCGUC
     1057    CUGCCUU CUGAUGAGGCCGAAAGGCCGAA AACUCGU
     1083    GGUGUUG CUGAUGAGGCCGAAAGGCCGAA AGCCCCA
     1084    UGGUGUU CUGAUGAGGCCGAAAGGCCGAA AAGCCCC
 5   1102    CUUGGAA CUGAUGAGGCCGAAAGGCCGAA AUUUCCU
     1104    CUCUUGG CUGAUGAGGCCGAAAGGCCGAA AGAUUUC
     1105    CCUCUUG CUGAUGAGGCCGAAAGGCCGAA AAGAUUU
     1114    CGCCGAC CUGAUGAGGCCGAAAGGCCGAA ACCUCUU
     1117    AGCCGCC CUGAUGAGGCCGAAAGGCCGAA ACAACCU
10   1125    GCUGGGG CUGAUGAGGCCGAAAGGCCGAA AGCCGCC
     1126    GGCUGGG CUGAUGAGGCCGAAAGGCCGAA AAGCCGC
     1144    GGACGGU CUGAUGAGGCCGAAAGGCCGAA ACUUGGG
     1150    GGCAGUG CUGAUGAGGCCGAAAGGCCGAA ACGGUGA
     1159    GCAUCUU CUGAUGAGGCCGAAAGGCCGAA AGGCAGU
15   1174    GGCAGGA CUGAUGAGGCCGAAAGGCCGAA AUCUUGG
     1176    UUGGCAG CUGAUGAGGCCGAAAGGCCGAA AGAUCUU
     1195    UGACCAC CUGAUGAGGCCGAAAGGCCGAA ACUCCCU
     1201    AAGAAUU CUGAUGAGGCCGAAAGGCCGAA ACCACGA
     1205    ACUGAAG CUGAUGAGGCCGAAAGGCCGAA AUUGACC
20   1206    CACUGAA CUGAUGAGGCCGAAAGGCCGAA AAUUGAC
     1208    AUCACUG CUGAUGAGGCCGAAAGGCCGAA AGAAUUG
     1209    CAUCACU CUGAUGAGGCCGAAAGGCCGAA AAGAAUU
     1224    AAAGAGG CUGAUGAGGCCGAAAGGCCGAA AUUUCAC
     1225    GAAAGAG CUGAUGAGGCCGAAAGGCCGAA AAUUUCA
25   1228    GUGGAAA CUGAUGAGGCCGAAAGGCCGAA AGGAAUU
     1230    GCGUGGA CUGAUGAGGCCGAAAGGCCGAA AGAGGAA
     1231    GGCGUGG CUGAUGAGGCCGAAAGGCCGAA AAGAGGA
     1232    GGGCGUG CUGAUGAGGCCGAAAGGCCGAA AAAGAGG
     1253    GCUACAG CUGAUGAGGCCGAAAGGCCGAA AUGUUGC
30   1254    AGCUACA CUGAUGAGGCCGAAAGGCCGAA AAUGUUG
     1258    UGUAAGC CUGAUGAGGCCGAAAGGCCGAA ACAGAAU
     1262    AAUGUGU CUGAUGAGGCCGAAAGGCCGAA AGCUACA
     1263    AAAUGUG CUGAUGAGGCCGAAAGGCCGAA AAGCUAC
     1269    CUCUUCA CUGAUGAGGCCGAAAGGCCGAA AUGUGUA
35   1270    CCUCUUC CUGAUGAGGCCGAAAGGCCGAA AAUGUGU
     1280    GUCACGA CUGAUGAGGCCGAAAGGCCGAA AUCCUCU
     1282    UAGUCAC CUGAUGAGGCCGAAAGGCCGAA AUAUCCU
```

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|      | 1289 | UGGACGG CUGAUGAGGCCGAAAGGCCGAA AGUCACG |
|------|------|----------------------------------------|
|      | 1294 | AGGCCUG CUGAUGAGGCCGAAAGGCCGAA ACGGUAG |
|      | 1302 | AGAAUAG CUGAUGAGGCCGAAAGGCCGAA AGGCCUG |
|      | 1305 | CUUAGAA CUGAUGAGGCCGAAAGGCCGAA AGGAGGC |
| 5    | 1307 | UUCUUAG CUGAUGAGGCCGAAAGGCCGAA AUAGGAG |
|      | 1308 | UUUCUUA CUGAUGAGGCCGAAAGGCCGAA AAUAGGA |
|      | 1310 | UUUUUCU CUGAUGAGGCCGAAAGGCCGAA AGAAUAG |
|      | 1321 | UUAAGAA CUGAUGAGGCCGAAAGGCCGAA AGCUUUU |
|      | 1323 | GCUUAAG CUGAUGAGGCCGAAAGGCCGAA AGAGCUU |
| 10   | 1324 | GGCUUAA CUGAUGAGGCCGAAAGGCCGAA AAGAGCU |
|      | 1326 | GAGGCUU CUGAUGAGGCCGAAAGGCCGAA AGAAGAG |
|      | 1327 | AGAGGCU CUGAUGAGGCCGAAAGGCCGAA AAGAAGA |
|      | 1333 | AAUCCAA CUGAUGAGGCCGAAAGGCCGAA AGGCUUA |
|      | 1335 | GAAAUCC CUGAUGAGGCCGAAAGGCCGAA AGAGGCU |
| 15   | 1340 | AUCUGGA CUGAUGAGGCCGAAAGGCCGAA AUCCAAG |
|      | 1341 | AAUCUGG CUGAUGAGGCCGAAAGGCCGAA AAUCCAA |
|      | 1342 | UAAUCUG CUGAUGAGGCCGAAAGGCCGAA AAAUCCA |
|      | 1348 | UUGGUGU CUGAUGAGGCCGAAAGGCCGAA AUCUGGA |
|      | 1349 | UUUGGUG CUGAUGAGGCCGAAAGGCCGAA AAUCUGG |
| 20   | 1363 | AGUUGGA CUGAUGAGGCCGAAAGGCCGAA ACAGUCU |
|      | 1364 | AAGUUGG CUGAUGAGGCCGAAAGGCCGAA AACAGUC |
|      | 1365 | CAAGUUG CUGAUGAGGCCGAAAGGCCGAA AAACAGU |
|      | 1371 | CUCAGUC CUGAUGAGGCCGAAAGGCCGAA AGUUGGA |
|      | 1386 | GGACUCG CUGAUGAGGCCGAAAGGCCGAA AGCUGCU |
| 25   | 1392 | CUGGAUG CUGAUGAGGCCGAAAGGCCGAA ACUCGGA |
|      | 1396 | AGCUCUG CUGAUGAGGCCGAAAGGCCGAA AUGGACU |
|      | 1404 | CUGCAGG CUGAUGAGGCCGAAAGGCCGAA AGCUCUG |
|      | 1405 | ACUGCAG CUGAUGAGGCCGAAAGGCCGAA AAGCUCU |
|      | 1413 | GAUCAUU CUGAUGAGGCCGAAAGGCCGAA ACUGCAG |
| 30   | 1420 | CAGCGGU CUGAUGAGGCCGAAAGGCCGAA AUCAUUG |
|      | 1435 | CCUCAGG CUGAUGAGGCCGAAAGGCCGAA AUGCCCA |
|      | 1444 | GAGACAU CUGAUGAGGCCGAAAGGCCGAA ACCUCAG |
|      | 1449 | GAGCCGA CUGAUGAGGCCGAAAGGCCGAA ACAUGAC |
|      | 1451 | UCGAGCC CUGAUGAGGCCGAAAGGCCGAA AGACAUG |
| 35   | 1456 | CUACCUC CUGAUGAGGCCGAAAGGCCGAA AGCCGAG |
|      | 1462 | UAAACAC CUGAUGAGGCCGAAAGGCCGAA ACCUCGA |
|      | 1467 | GGCUGUA CUGAUGAGGCCGAAAGGCCGAA ACACUAC |

|    |      |         |                               |         |
|----|------|---------|-------------------------------|---------|
|    | 1468 | GGGCUGU | CUGAUGAGGCCGAAAGGCCGAA | AACACUA |
|    | 1469 | AGGGCUG | CUGAUGAGGCCGAAAGGCCGAA | AAACACU |
|    | 1477 | UGUUCAU | CUGAUGAGGCCGAAAGGCCGAA | AGGGCUG |
|    | 1501 | UGUCGAA | CUGAUGAGGCCGAAAGGCCGAA | AGGCUCA |
| 5  | 1503 | GAUGUCG | CUGAUGAGGCCGAAAGGCCGAA | AGAGGCU |
|    | 1504 | UGAUGUC | CUGAUGAGGCCGAAAGGCCGAA | AAGAGGC |
|    | 1510 | GGUUGAU | CUGAUGAGGCCGAAAGGCCGAA | AUGUCGA |
|    | 1513 | CAGGGUU | CUGAUGAGGCCGAAAGGCCGAA | AUGAUGU |
|    | 1525 | GAGUGAU | CUGAUGAGGCCGAAAGGCCGAA | AUCUCAG |
| 10 | 1526 | CGAGUGA | CUGAUGAGGCCGAAAGGCCGAA | AAUCUCA |
|    | 1528 | CUCGAGU | CUGAUGAGGCCGAAAGGCCGAA | AUAAUCU |
|    | 1532 | CCAUCUC | CUGAUGAGGCCGAAAGGCCGAA | AGUGAUA |
|    | 1542 | CAGCAGG | CUGAUGAGGCCGAAAGGCCGAA | AGCCAUC |
|    | 1543 | GCAGCAG | CUGAUGAGGCCGAAAGGCCGAA | AAGCCAU |
| 15 | 1563 | GAAGCCA | CUGAUGAGGCCGAAAGGCCGAA | AGUCCAU |
|    | 1564 | GGAAGCC | CUGAUGAGGCCGAAAGGCCGAA | AAGUCCA |
|    | 1569 | CUCAGGG | CUGAUGAGGCCGAAAGGCCGAA | AGCCAAA |
|    | 1570 | GCUCAGG | CUGAUGAGGCCGAAAGGCCGAA | AAGCCAA |
|    | 1592 | UGGAGGA | CUGAUGAGGCCGAAAGGCCGAA | AUCCACC |
| 20 | 1593 | CUGGAGG | CUGAUGAGGCCGAAAGGCCGAA | AAUCCAC |
|    | 1594 | UCUGGAG | CUGAUGAGGCCGAAAGGCCGAA | AAAUCCA |
|    | 1597 | AGCUCUG | CUGAUGAGGCCGAAAGGCCGAA | AGGAAAU |
|    | 1605 | CUAGCUC | CUGAUGAGGCCGAAAGGCCGAA | AGCUCUG |
|    | 1611 | AGACUUC | CUGAUGAGGCCGAAAGGCCGAA | AGCUCAA |
| 25 | 1617 | CCUUGGA | CUGAUGAGGCCGAAAGGCCGAA | ACUUCUA |
|    | 1619 | CUCCUUG | CUGAUGAGGCCGAAAGGCCGAA | AGACUUC |
|    | 1629 | CCAUCCC | CUGAUGAGGCCGAAAGGCCGAA | ACCUCCU |
|    | 1641 | CUGCUAC | CUGAUGAGGCCGAAAGGCCGAA | AGCCCCA |
|    | 1644 | CUUCUGC | CUGAUGAGGCCGAAAGGCCGAA | ACAAGCC |
| 30 | 1666 | CAGCUGU | CUGAUGAGGCCGAAAGGCCGAA | AGCCUGG |
|    | 1686 | UGGAGGA | CUGAUGAGGCCGAAAGGCCGAA | ACACCAG |
|    | 1688 | GCUGGAG | CUGAUGAGGCCGAAAGGCCGAA | AGACACC |
|    | 1691 | CACGCUG | CUGAUGAGGCCGAAAGGCCGAA | AGGAGAC |
|    | 1707 | CUAACCC | CUGAUGAGGCCGAAAGGCCGAA | ACUUCCA |
| 35 | 1712 | UACUCCU | CUGAUGAGGCCGAAAGGCCGAA | ACCCAAC |
|    | 1713 | GUACUCC | CUGAUGAGGCCGAAAGGCCGAA | AACCCAA |
|    | 1719 | AUCUCCG | CUGAUGAGGCCGAAAGGCCGAA | ACUCCUA |

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| 1733 | GGGAGCC | CUGAUGAGGCCGAAAGGCCGAA | AUCUCCA |
|------|---------|------------------------|--------|
| 1738 | GAGUUGG | CUGAUGAGGCCGAAAGGCCGAA | AGCCAAU |
| 1745 | UAGGGAG | CUGAUGAGGCCGAAAGGCCGAA | AGUUGGG |
| 1748 | GGAUAGG | CUGAUGAGGCCGAAAGGCCGAA | AGGAGUU |
| 1752 | UUUAGGA | CUGAUGAGGCCGAAAGGCCGAA | AGGGAGG |
| 1754 | CCUUUAG | CUGAUGAGGCCGAAAGGCCGAA | AUAGGGA |
| 1757 | GGGCCUU | CUGAUGAGGCCGAAAGGCCGAA | AGGAUAG |
| 1773 | GCACUUU | CUGAUGAGGCCGAAAGGCCGAA | AUGCCAG |
| 1774 | AGCACUU | CUGAUGAGGCCGAAAGGCCGAA | AAUGCCA |


Table IV: Rabbit CETP HH Target Sequence

| nt. Position | Target Sequence | nt. Position | Target Sequence |
|--------------|-----------------|--------------|-----------------|
| 20 | GGCgCCU C cuACGAG | 305 | uAcAgcU A cACGaGu |
| 23 | GCCUcCU a CgAgGcu | 305 | UAcaGCU A CACgAgU |
| 23 | gccUCCU a CgagGCU | 305 | UaCAgCU A CACgAgU |
| 36 | CuGGCAU C GUGUGuC | 323 | UGGggGU U GGGcauc |
| 43 | cgUGuGU c GCAucAC | 330 | UGGGcAU c aAUCAGU |
| 43 | CgUgUgU c gCAUCAc | 334 | CauCAaU C AGucuGU |
| 48 | GuCGCAU C ACCAAGC | 334 | cAUcaAU C AGUCugU |
| 63 | CcGCCCU C uUGGUGU | 338 | aAUCAGU C ugUcGAC |
| 71 | uUGGUGU U GAACCAa | 342 | AGUCugU c GACUUCG |
| 96 | AGGUGgU C CAGACgG | 342 | AgUCUGU c GacUuCg |
| 96 | AGGUGgU C CaGaCgg | 347 | gUcGACU U CGAGAUC |
| 96 | AGGuGGU C caGAcGG | 348 | UcGACUU C GAGAUCG |
| 107 | ACgGCCU U CCAGCGc | 354 | UCGAGAU C GACUCUG |
| 108 | CgGCCUU C CAGCGcG | 354 | UCGaGaU C gAcUCUg |
| 122 | GCCgGCU A uCCgGAc | 354 | UCGAGaU c GacUCUg |
| 132 | CgGAcgU C AgcGGCG | 354 | UcGAGAU c GaCUCug |
| 132 | cgGAcGU c agCGGCG | 359 | AuCgACU c ugCCAUu |
| 132 | cGGacGU C aGCGGCg | 359 | AUCGACU C UGCCAUU |
| 156 | UGAUGCU C CUcGGCC | 359 | AuCGAcU C UgCCAuU |
| 159 | UGCUCCU c GGCCggG | 366 | CUGCCAU U GACCUCC |
| 168 | GCCggGU C AAGUAcG | 372 | UUGACCU C CAGAUCA |
| 168 | GccGGGU c AaGuacg | 372 | UuGAccU C CAGAUcA |
| 173 | GUCAAGU A cGGGcUG | 372 | UUGAcCU c cAgaUCa |
| 189 | aCaACcU c CAgAuCA | 378 | UCCAGAU C AACACAg |

|  | 189 | ACAACcU C CAGAUCA | 378 | UCCAGAU c AacaCAg |
|  | 195 | UCCAGAU C AGCCACc | 378 | UCCAgaU c aACacAG |
|  | 206 | CACcUGU C CAUCGCC | 434 | GACUGCU A CCUGgCU |
|  | 210 | UGUCCAU C GCCAGCA | 434 | gACUgCU a CCUggCu |
| 5 | 249 | AGaCCAU c GAcGUCg | 442 | cCUGGcU u UccAUaA |
|  | 255 | UcGAcGU C gCCAUcC | 442 | CCUGgCU U UCCAUAA |
|  | 261 | UCgCCAU c CAGAACG | 442 | CCUggCU u UCCauAA |
|  | 261 | ucgCCAU C CAGAaCg | 443 | CUGgCUU U CCAUAAa |
|  | 272 | aACgUgU c CGuGgUC | 443 | CugGcUU u CcauAAA |
| 10 | 272 | AACGUGU C cGUGGUC | 444 | uGGcuuU c CAUAaAC |
|  | 279 | CcGUGGU C UUCAAGG | 444 | UGgCUUU C CAUAAaC |
|  | 279 | ccGugGU C UuCAAGG | 448 | UUUCCAU A AaCUGCU |
|  | 281 | GUGGUCU U CAAGGGG | 456 | AaCUGCU C CUGCAcC |
|  | 282 | UGGUCUU C AAGGGGA | 465 | UGCAcCU C CAgGGGG |
| 15 | 299 | CUGAAcU A caGCUAC | 492 | gGUGgCU C aaGcAGc |
|  | 299 | CuGAaCU a cAGCUAc | 492 | GGUGGcU C AAGCAGC |
|  | 492 | GGuGGCU c aagCAGC | 738 | AGGcCuU C CCCCUCC |
|  | 492 | gGUgGCU c AagCAgc | 744 | UCCCCCU C CgCgCCU |
|  | 503 | CAGCUcU U CACAAAc | 752 | CgCgCCU U CcCGCCC |
| 20 | 503 | CAgcUCU U CAcaaAc | 752 | CGcGcCU u CCcgCCc |
|  | 504 | aGCucuU c ACAaACu | 753 | gCgCCUU C cCGCCCg |
|  | 504 | AGCUcUU C ACAAAcU | 776 | GGGGACU C CCGCAUG |
|  | 512 | ACAAAcU U CAUCUCC | 788 | AUGCUcU A CUUCUGG |
|  | 513 | CAAAcUU C AUCUCCU | 791 | CUcUACU U CUGGUUC |
| 25 | 516 | AcUUCAU C UCCUUCA | 792 | UcUACUU C UGGUUCU |
|  | 518 | UUCAUCU C CUUCACC | 797 | UUCUGGU U CUCcGAu |
|  | 521 | AUCUCCU U CACCCUG | 798 | UCUGGUU C UCcGAuC |
|  | 521 | aUCUcCU U cAcCCUg | 800 | UGGUUCU C cGAuCaA |
|  | 521 | aUCUCCU u CAcCcUG | 805 | CUCcGaU c aAGUGCu |
| 30 | 522 | UCUCCUU C ACCCUGA | 818 | cUCaACU C cCUGGCC |
|  | 537 | AGCUGaU u CUGAAGc | 836 | GccGCcU U CCAGGAg |
|  | 552 | GACAGgU C UGCAAuG | 836 | GcCgccU u cCAGgAG |
|  | 552 | GaCAggU C UgCAaUG | 837 | CCgCCUU c CAGgaGG |
|  | 564 | AuGAGAU C AACacCA | 837 | ccGCcUU C CAGGAgG |
| 35 | 573 | ACacCAU C UCcAACA | 852 | gCCgUCU C GugCuCA |
|  | 573 | AcacCAU C UcCAAcA | 852 | GCCGuCU C gUGCUCA |
|  | 575 | acCAUCU C cAACAUC | 858 | UCgUGCU C AGCCUGA |

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| | 582 | cCAACaU C AUggCUg | 878 | GAuGAGU U CAAGaaA |
|---|------|-------------------|-----|------------------|
| | 582 | CcAACAU C AUGGCuG | 879 | AuGAGUU C AAGaaAG |
| | 593 | GCuGAcU U UGUCCAG | 905 | caGGGuU U CgACACC |
| | 594 | CuGAcUU U GUCCAGA | 906 | aGGGuUU C gACACCA |
| 5 | 597 | AcUUUGU C CAGACgA | 924 | AGGAAAU C UUCCAgG |
| | 618 | CCAGCAU C CUcUCAG | 926 | GAAAUCU U CCAgGAG |
| | 621 | GCAUCCU c UCAGAUG | 926 | GAAaUCU u CcAGGAG |
| | 623 | AUCCUcU C AGAUGGA | 927 | AAAUCUU C CAgGAGc |
| | 623 | aUccUCU c AGAuggA | 936 | aGgAGCU U UCCAGag |
| 10 | 636 | GAGACAU c GGGGUGG | 937 | gGAGCUU u CcagAGg |
| | 648 | UGGACAU U UCCgUGA | 969 | CCcAGGU A GCcgUCC |
| | 649 | GGACAUU U CCgUGAC | 969 | CCCAgGU a gCCGUCC |
| | 650 | GACAUUU C CgUGACg | 975 | UagCCGU C CACUGCC |
| | 650 | gAcauUU C cGUGAcG | 984 | ACUGCCU u AAGgUGC |
| 15 | 669 | ccCCuGU C AUCACAG | 985 | cuGCcUU A AgGUGCc |
| | 672 | CuGUCAU C ACAGCCa | 999 | CCAAGAU C UCCUGCC |
| | 672 | cuGUCAU c aCAgCcA | 1001 | AAGAUCU C CUGCCAg |
| | 683 | GCCAccU a CcUgGAg | 1020 | gGGGuGU C GUGGUgu |
| | 683 | GCCaCCU A CCUGGAG | 1028 | gUGGUGU C UuCUuCc |
| 20 | 692 | CuggAgU c cCAUCaC | 1030 | GGUgucU U CUUCcGU |
| | 692 | CUGGAGU C CCAUCAC | 1030 | GGUGUCU u CUuCcGu |
| | 697 | GUCCCAU C ACAAGGG | 1034 | UCuUcUU C CGUcGcc |
| | 706 | CAAGGGU C AcUUCAc | 1049 | GUGAcgU U CCgCUUc |
| | 710 | GGUCAcU U CAcgcAC | 1049 | gUGaCgU u CcGCUuC |
| 25 | 711 | GUCAcUU C AcgcACA | 1050 | UGAcgUU C CgCUUcC |
| | 726 | AGAAcGU C UCcGAGG | 1055 | UUCCgCU U cCCcCGC |
| | 728 | AAcGUCU C cGAGGcC | 1056 | UCCgCUU c CCcCGCC |
| | 737 | GAGgCcU u CcCcCuC | 1088 | GUgGCcU A CAggUUU |
| | 1094 | UACAggU U UGAgGAG | 1408 | CGAGauU a uCACUCu |
| 30 | 1095 | ACAggUU U GAgGAGG | 1408 | cGAGAUU A UCACUCu |
| | 1105 | gGAGGAU A UCaUcAC | 1410 | AGAUUAU C ACUCucG |
| | 1107 | AGGAUAU C aUcACcA | 1414 | UAUCACU C ucGAUGG |
| | 1110 | auAUcAU C ACCaCcG | 1445 | AUGGACU U cGGuUUu |
| | 1119 | CCACcGU C CaGgCCu | 1446 | UGGACUU c GGuUUuC |
| 35 | 1119 | CcACCGU C CAGGCCU | 1451 | UUcGGuU U uCCcaAG |
| | 1119 | CcAcCgU c cAGGCCu | 1452 | UcGGuUU u CCcaAGC |
| | 1127 | cagGCCU C cUacUCC | 1474 | GGugGAU U UccUgCA |

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| | 1127 | CAGGCCU C CUAcUCc | 1474 | GGUGGAU U UCCUgCA |
| | 1130 | GCCUCCU A cUCccAG | 1475 | GUGGAUU U CCUgCAG |
| | 1133 | UCCUAcU C ccAGAAA | 1476 | UGGAUUU C CUgCAGA |
| | 1146 | AAaagCU C UUcCuAC | 1529 | gACGuCU C cGcCCAu |
| 5 | 1146 | AAAAGCU C UUCcUAc | 1529 | GAcGUCU C CgccCAu |
| | 1148 | AAGCUCU U CcUAcaC | 1549 | UgGagGU c aGGgagU |
| | 1149 | AGCUCUU C cUAcaCC | 1580 | GAUGGCU c CCaaCUc |
| | 1152 | UCUUCcU A caCCUCU | 1580 | gaUGGCU C CCAACUC |
| | 1158 | UAcaCCU C UUGGAUU | 1587 | CCCAACU C CUuCugu |
| 10 | 1160 | caCCUCU U GGAUUUC | 1595 | CUucUGU c CuGaaGa |
| | 1165 | CuUGgAU u UCCAguG | 1595 | CUuCUgU c CUgAagA |
| | 1165 | CUUGGAU U UCCAGug | 1595 | cuuCUgU C CUgAAGa |
| | 1166 | UUGGAUU U CCAGugc | 1624 | GCAgCAU a CccUgGg |
| | 1166 | UUGGaUU u ccAGUgC | 1694 | uCcGGaU C cCAGCUG |
| 15 | 1245 | AGgCUGU U UCCAACc | 1787 | cCuGGCU u uAGcCUG |
| | 1246 | GgCUGUU U CCAACcU | 1788 | CUGGCuU U AgccUGC |
| | 1247 | gCuGuuU C CaACCUG | 1816 | gCuAaAU c UCuCuGG |
| | 1247 | gCUGUUU C CAACcUG | 1818 | UaAAUCU C UcuGGCu |
| | 1247 | gcUGUUU c CAaCCug | 1818 | uAAaucU C UcUgGCU |
| 20 | 1268 | AGCcGCU C CGAGUCC | 1828 | UggCUGU C UcUCucU |
| | 1274 | UCCGAGU C CcUgCAG | 1847 | CUcaAGU a AAcGAau |
| | 1286 | CAGAGCU c uCUcCgc | | |
| | 1302 | cCCUGAU c gCCAcGg | | |
| | 1317 | UGGGCAU C CCgGAGG | | |
| 25 | 1326 | CgGAGGU C AUGUCUC | | |
| | 1331 | GUCAUGU C UCGGCUC | | |
| | 1333 | CAUGUCU C GGCUCGA | | |
| | 1338 | CUCGGCU C GAGGUgG | | |
| | 1349 | GuGGCgU u CaCAGCC | | |
| 30 | 1349 | GUgGcGU U cACAGCC | | |
| | 1350 | UgGcGUU c ACAGCCC | | |
| | 1350 | UGGcGuU c acAGCcc | | |
| | 1359 | CAGCCCU C AUGAACA | | |
| | 1383 | UGgaCCU C UUCGAaA | | |
| 35 | 1385 | gaCCUCU U CGAaAUC | | |
| | 1386 | aCCUCUU C GAaAUCA | | |
| | 1392 | UCGAaAU C AUCAACC | | |

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1395    AaAUCAU C AACCCcG
1407    CcGAGAU U AUCACUC
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Table V:   Rabbit CETP Hammerhead Ribozyme Sequence

nt.

| Position | Ribozyme Sequence |
|---|---|
| 20 | CUCGUAG CUGAUGAGGCCGAAAGGCCGAA AGGCGCC |
| 23 | AGCCUCG CUGAUGAGGCCGAAAGGCCGAA AGGAGGC |
| 23 | AGCCUCG CUGAUGAGGCCGAAAGGCCGAA AGGAGGC |
| 36 | GACACAC CUGAUGAGGCCGAAAGGCCGAA AUGCCAG |
| 43 | GUGAUGC CUGAUGAGGCCGAAAGGCCGAA ACACACG |
| 43 | GUGAUGC CUGAUGAGGCCGAAAGGCCGAA ACACACG |
| 48 | GCUUGGU CUGAUGAGGCCGAAAGGCCGAA AUGCGAC |
| 63 | ACACCAA CUGAUGAGGCCGAAAGGCCGAA AGGGCGG |
| 71 | UUGGUUC CUGAUGAGGCCGAAAGGCCGAA ACACCAA |
| 96 | CCGUCUG CUGAUGAGGCCGAAAGGCCGAA ACCACCU |
| 96 | CCGUCUG CUGAUGAGGCCGAAAGGCCGAA ACCACCU |
| 96 | CCGUCUG CUGAUGAGGCCGAAAGGCCGAA ACCACCU |
| 107 | GCGCUGG CUGAUGAGGCCGAAAGGCCGAA AGGCCGU |
| 108 | CGCGCUG CUGAUGAGGCCGAAAGGCCGAA AAGGCCG |
| 122 | GUCCGGA CUGAUGAGGCCGAAAGGCCGAA AGCCGGC |
| 132 | CGCCGCU CUGAUGAGGCCGAAAGGCCGAA ACGUCCG |
| 132 | CGCCGCU CUGAUGAGGCCGAAAGGCCGAA ACGUCCG |
| 132 | CGCCGCU CUGAUGAGGCCGAAAGGCCGAA ACGUCCG |
| 156 | GGCCGAG CUGAUGAGGCCGAAAGGCCGAA AGCAUCA |
| 159 | CCCGGCC CUGAUGAGGCCGAAAGGCCGAA AGGAGCA |
| 168 | CGUACUU CUGAUGAGGCCGAAAGGCCGAA ACCCGGC |
| 168 | CGUACUU CUGAUGAGGCCGAAAGGCCGAA ACCCGGC |
| 173 | CAGCCCG CUGAUGAGGCCGAAAGGCCGAA ACUUGAC |
| 189 | UGAUCUG CUGAUGAGGCCGAAAGGCCGAA AGGUUGU |
| 189 | UGAUCUG CUGAUGAGGCCGAAAGGCCGAA AGGUUGU |
| 195 | GGUGGCU CUGAUGAGGCCGAAAGGCCGAA AUCUGGA |
| 206 | GGCGAUG CUGAUGAGGCCGAAAGGCCGAA ACAGGUG |
| 210 | UGCUGGC CUGAUGAGGCCGAAAGGCCGAA AUGGACA |
| 249 | CGACGUC CUGAUGAGGCCGAAAGGCCGAA AUGGUCU |
| 255 | GGAUGGC CUGAUGAGGCCGAAAGGCCGAA ACGUCGA |
| 261 | CGUUCUG CUGAUGAGGCCGAAAGGCCGAA AUGGCGA |

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         261      CGUUCUG  CUGAUGAGGCCGAAAGGCCGAA  AUGGCGA
         272      GACCACG  CUGAUGAGGCCGAAAGGCCGAA  ACACGUU
         272      GACCACG  CUGAUGAGGCCGAAAGGCCGAA  ACACGUU
         279      CCUUGAA  CUGAUGAGGCCGAAAGGCCGAA  ACCACGG
    5    279      CCUUGAA  CUGAUGAGGCCGAAAGGCCGAA  ACCACGG
         281      CCCCUUG  CUGAUGAGGCCGAAAGGCCGAA  AGACCAC
         282      UCCCCUU  CUGAUGAGGCCGAAAGGCCGAA  AAGACCA
         299      GUAGCUG  CUGAUGAGGCCGAAAGGCCGAA  AGUUCAG
         299      GUAGCUG  CUGAUGAGGCCGAAAGGCCGAA  AGUUCAG
   10    305      ACUCGUG  CUGAUGAGGCCGAAAGGCCGAA  AGCUGUA
         305      ACUCGUG  CUGAUGAGGCCGAAAGGCCGAA  AGCUGUA
         305      ACUCGUG  CUGAUGAGGCCGAAAGGCCGAA  AGCUGUA
         323      GAUGCCC  CUGAUGAGGCCGAAAGGCCGAA  ACCCCCA
         330      ACUGAUU  CUGAUGAGGCCGAAAGGCCGAA  AUGCCCA
   15    334      ACAGACU  CUGAUGAGGCCGAAAGGCCGAA  AUUGAUG
         334      ACAGACU  CUGAUGAGGCCGAAAGGCCGAA  AUUGAUG
         338      GUCGACA  CUGAUGAGGCCGAAAGGCCGAA  ACUGAUU
         342      CGAAGUC  CUGAUGAGGCCGAAAGGCCGAA  ACAGACU
         342      CGAAGUC  CUGAUGAGGCCGAAAGGCCGAA  ACAGACU
   20    347      GAUCUCG  CUGAUGAGGCCGAAAGGCCGAA  AGUCGAC
         348      CGAUCUC  CUGAUGAGGCCGAAAGGCCGAA  AAGUCGA
         354      CAGAGUC  CUGAUGAGGCCGAAAGGCCGAA  AUCUCGA
         354      CAGAGUC  CUGAUGAGGCCGAAAGGCCGAA  AUCUCGA
         354      CAGAGUC  CUGAUGAGGCCGAAAGGCCGAA  AUCUCGA
   25    354      CAGAGUC  CUGAUGAGGCCGAAAGGCCGAA  AUCUCGA
         359      AAUGGCA  CUGAUGAGGCCGAAAGGCCGAA  AGUCGAU
         359      AAUGGCA  CUGAUGAGGCCGAAAGGCCGAA  AGUCGAU
         359      AAUGGCA  CUGAUGAGGCCGAAAGGCCGAA  AGUCGAU
         366      GGAGGUC  CUGAUGAGGCCGAAAGGCCGAA  AUGGCAG
   30    372      UGAUCUG  CUGAUGAGGCCGAAAGGCCGAA  AGGUCAA
         372      UGAUCUG  CUGAUGAGGCCGAAAGGCCGAA  AGGUCAA
         372      UGAUCUG  CUGAUGAGGCCGAAAGGCCGAA  AGGUCAA
         378      CUGUGUU  CUGAUGAGGCCGAAAGGCCGAA  AUCUGGA
         378      CUGUGUU  CUGAUGAGGCCGAAAGGCCGAA  AUCUGGA
   35    378      CUGUGUU  CUGAUGAGGCCGAAAGGCCGAA  AUCUGGA
         434      AGCCAGG  CUGAUGAGGCCGAAAGGCCGAA  AGCAGUC
         434      AGCCAGG  CUGAUGAGGCCGAAAGGCCGAA  AGCAGUC
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|    |     |         |                         |         |
|----|-----|---------|-------------------------|---------|
|    | 442 | UUAUGGA | CUGAUGAGGCCGAAAGGCCGAA | AGCCAGG |
|    | 442 | UUAUGGA | CUGAUGAGGCCGAAAGGCCGAA | AGCCAGG |
|    | 442 | UUAUGGA | CUGAUGAGGCCGAAAGGCCGAA | AGCCAGG |
|    | 443 | UUUAUGG | CUGAUGAGGCCGAAAGGCCGAA | AAGCCAG |
| 5  | 443 | UUUAUGG | CUGAUGAGGCCGAAAGGCCGAA | AAGCCAG |
|    | 444 | GUUUAUG | CUGAUGAGGCCGAAAGGCCGAA | AAAGCCA |
|    | 444 | GUUUAUG | CUGAUGAGGCCGAAAGGCCGAA | AAAGCCA |
|    | 448 | AGCAGUU | CUGAUGAGGCCGAAAGGCCGAA | AUGGAAA |
|    | 456 | GGUGCAG | CUGAUGAGGCCGAAAGGCCGAA | AGCAGUU |
| 10 | 465 | CCCCCUG | CUGAUGAGGCCGAAAGGCCGAA | AGGUGCA |
|    | 492 | GCUGCUU | CUGAUGAGGCCGAAAGGCCGAA | AGCCACC |
|    | 492 | GCUGCUU | CUGAUGAGGCCGAAAGGCCGAA | AGCCACC |
|    | 492 | GCUGCUU | CUGAUGAGGCCGAAAGGCCGAA | AGCCACC |
|    | 492 | GCUGCUU | CUGAUGAGGCCGAAAGGCCGAA | AGCCACC |
| 15 | 503 | GUUUGUG | CUGAUGAGGCCGAAAGGCCGAA | AGAGCUG |
|    | 503 | GUUUGUG | CUGAUGAGGCCGAAAGGCCGAA | AGAGCUG |
|    | 504 | AGUUUGU | CUGAUGAGGCCGAAAGGCCGAA | AAGAGCU |
|    | 504 | AGUUUGU | CUGAUGAGGCCGAAAGGCCGAA | AAGAGCU |
|    | 512 | GGAGAUG | CUGAUGAGGCCGAAAGGCCGAA | AGUUUGU |
| 20 | 513 | AGGAGAU | CUGAUGAGGCCGAAAGGCCGAA | AAGUUUG |
|    | 516 | UGAAGGA | CUGAUGAGGCCGAAAGGCCGAA | AUGAAGU |
|    | 518 | GGUGAAG | CUGAUGAGGCCGAAAGGCCGAA | AGAUGAA |
|    | 521 | CAGGGUG | CUGAUGAGGCCGAAAGGCCGAA | AGGAGAU |
|    | 521 | CAGGGUG | CUGAUGAGGCCGAAAGGCCGAA | AGGAGAU |
| 25 | 521 | CAGGGUG | CUGAUGAGGCCGAAAGGCCGAA | AGGAGAU |
|    | 522 | UCAGGGU | CUGAUGAGGCCGAAAGGCCGAA | AAGGAGA |
|    | 537 | GCUUCAG | CUGAUGAGGCCGAAAGGCCGAA | AUCAGCU |
|    | 552 | CAUUGCA | CUGAUGAGGCCGAAAGGCCGAA | ACCUGUC |
|    | 552 | CAUUGCA | CUGAUGAGGCCGAAAGGCCGAA | ACCUGUC |
| 30 | 564 | UGGUGUU | CUGAUGAGGCCGAAAGGCCGAA | AUCUCAU |
|    | 573 | UGUUGGA | CUGAUGAGGCCGAAAGGCCGAA | AUGGUGU |
|    | 573 | UGUUGGA | CUGAUGAGGCCGAAAGGCCGAA | AUGGUGU |
|    | 575 | GAUGUUG | CUGAUGAGGCCGAAAGGCCGAA | AGAUGGU |
|    | 582 | CAGCCAU | CUGAUGAGGCCGAAAGGCCGAA | AUGUUGG |
| 35 | 582 | CAGCCAU | CUGAUGAGGCCGAAAGGCCGAA | AUGUUGG |
|    | 593 | CUGGACA | CUGAUGAGGCCGAAAGGCCGAA | AGUCAGC |
|    | 594 | UCUGGAC | CUGAUGAGGCCGAAAGGCCGAA | AAGUCAG |

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     597    UCGUCUG CUGAUGAGGCCGAAAGGCCGAA ACAAAGU
     618    CUGAGAG CUGAUGAGGCCGAAAGGCCGAA AUGCUGG
     621    CAUCUGA CUGAUGAGGCCGAAAGGCCGAA AGGAUGC
     623    UCCAUCU CUGAUGAGGCCGAAAGGCCGAA AGAGGAU
  5  623    UCCAUCU CUGAUGAGGCCGAAAGGCCGAA AGAGGAU
     636    CCACCCC CUGAUGAGGCCGAAAGGCCGAA AUGUCUC
     648    UCACGGA CUGAUGAGGCCGAAAGGCCGAA AUGUCCA
     649    GUCACGG CUGAUGAGGCCGAAAGGCCGAA AAUGUCC
     650    CGUCACG CUGAUGAGGCCGAAAGGCCGAA AAAUGUC
 10  650    CGUCACG CUGAUGAGGCCGAAAGGCCGAA AAAUGUC
     669    CUGUGAU CUGAUGAGGCCGAAAGGCCGAA ACAGGGG
     672    UGGCUGU CUGAUGAGGCCGAAAGGCCGAA AUGACAG
     672    UGGCUGU CUGAUGAGGCCGAAAGGCCGAA AUGACAG
     683    CUCCAGG CUGAUGAGGCCGAAAGGCCGAA AGGUGGC
 15  683    CUCCAGG CUGAUGAGGCCGAAAGGCCGAA AGGUGGC
     692    GUGAUGG CUGAUGAGGCCGAAAGGCCGAA ACUCCAG
     692    GUGAUGG CUGAUGAGGCCGAAAGGCCGAA ACUCCAG
     697    CCCUUGU CUGAUGAGGCCGAAAGGCCGAA AUGGGAC
     706    GUGAAGU CUGAUGAGGCCGAAAGGCCGAA ACCCUUG
 20  710    GUGCGUG CUGAUGAGGCCGAAAGGCCGAA AGUGACC
     711    UGUGCGU CUGAUGAGGCCGAAAGGCCGAA AAGUGAC
     726    CCUCGGA CUGAUGAGGCCGAAAGGCCGAA ACGUUCU
     728    GGCCUCG CUGAUGAGGCCGAAAGGCCGAA AGACGUU
     737    GAGGGGG CUGAUGAGGCCGAAAGGCCGAA AGGCCUC
 25  738    GGAGGGG CUGAUGAGGCCGAAAGGCCGAA AAGGCCU
     744    AGGCGCG CUGAUGAGGCCGAAAGGCCGAA AGGGGGA
     752    GGGCGGG CUGAUGAGGCCGAAAGGCCGAA AGGCGCG
     752    GGGCGGG CUGAUGAGGCCGAAAGGCCGAA AGGCGCG
     753    CGGGCGG CUGAUGAGGCCGAAAGGCCGAA AAGGCGC
 30  776    CAUGCGG CUGAUGAGGCCGAAAGGCCGAA AGUCCCC
     788    CCAGAAG CUGAUGAGGCCGAAAGGCCGAA AGAGCAU
     791    GAACCAG CUGAUGAGGCCGAAAGGCCGAA AGUAGAG
     792    AGAACCA CUGAUGAGGCCGAAAGGCCGAA AAGUAGA
     797    AUCGGAG CUGAUGAGGCCGAAAGGCCGAA ACCAGAA
 35  798    GAUCGGA CUGAUGAGGCCGAAAGGCCGAA AACCAGA
     800    UUGAUCG CUGAUGAGGCCGAAAGGCCGAA AGAACCA
     805    AGCACUU CUGAUGAGGCCGAAAGGCCGAA AUCGGAG
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        818    GGCCAGG CUGAUGAGGCCGAAAGGCCGAA AGUUGAG
        836    CUCCUGG CUGAUGAGGCCGAAAGGCCGAA AGGCGGC
        836    CUCCUGG CUGAUGAGGCCGAAAGGCCGAA AGGCGGC
        837    CCUCCUG CUGAUGAGGCCGAAAGGCCGAA AAGGCGG
  5     837    CCUCCUG CUGAUGAGGCCGAAAGGCCGAA AAGGCGG
        852    UGAGCAC CUGAUGAGGCCGAAAGGCCGAA AGACGGC
        852    UGAGCAC CUGAUGAGGCCGAAAGGCCGAA AGACGGC
        858    UCAGGCU CUGAUGAGGCCGAAAGGCCGAA AGCACGA
        878    UUUCUUG CUGAUGAGGCCGAAAGGCCGAA ACUCAUC
 10     879    CUUUCUU CUGAUGAGGCCGAAAGGCCGAA AACUCAU
        905    GGUGUCG CUGAUGAGGCCGAAAGGCCGAA AACCCUG
        906    UGGUGUC CUGAUGAGGCCGAAAGGCCGAA AAACCCU
        924    CCUGGAA CUGAUGAGGCCGAAAGGCCGAA AUUUCCU
        926    CUCCUGG CUGAUGAGGCCGAAAGGCCGAA AGAUUUC
 15     926    CUCCUGG CUGAUGAGGCCGAAAGGCCGAA AGAUUUC
        927    GCUCCUG CUGAUGAGGCCGAAAGGCCGAA AAGAUUU
        936    CUCUGGA CUGAUGAGGCCGAAAGGCCGAA AGCUCCU
        937    CCUCUGG CUGAUGAGGCCGAAAGGCCGAA AAGCUCC
        969    GGACGGC CUGAUGAGGCCGAAAGGCCGAA ACCUGGG
 20     969    GGACGGC CUGAUGAGGCCGAAAGGCCGAA ACCUGGG
        975    GGCAGUG CUGAUGAGGCCGAAAGGCCGAA ACGGCUA
        984    GCACCUU CUGAUGAGGCCGAAAGGCCGAA AGGCAGU
        985    GGCACCU CUGAUGAGGCCGAAAGGCCGAA AAGGCAG
        999    GGCAGGA CUGAUGAGGCCGAAAGGCCGAA AUCUUGG
 25    1001    CUGGCAG CUGAUGAGGCCGAAAGGCCGAA AGAUCUU
       1020    ACACCAC CUGAUGAGGCCGAAAGGCCGAA ACACCCC
       1028    GGAAGAA CUGAUGAGGCCGAAAGGCCGAA ACACCAC
       1030    ACGGAAG CUGAUGAGGCCGAAAGGCCGAA AGACACC
       1030    ACGGAAG CUGAUGAGGCCGAAAGGCCGAA AGACACC
 30    1034    GGCGACG CUGAUGAGGCCGAAAGGCCGAA AAGAAGA
       1049    GAAGCGG CUGAUGAGGCCGAAAGGCCGAA ACGUCAC
       1049    GAAGCGG CUGAUGAGGCCGAAAGGCCGAA ACGUCAC
       1050    GGAAGCG CUGAUGAGGCCGAAAGGCCGAA AACGUCA
       1055    GCGGGGG CUGAUGAGGCCGAAAGGCCGAA AGCGGAA
 35    1056    GGCGGGG CUGAUGAGGCCGAAAGGCCGAA AAGCGGA
       1088    AAACCUG CUGAUGAGGCCGAAAGGCCGAA AGGCCAC
       1094    CUCCUCA CUGAUGAGGCCGAAAGGCCGAA ACCUGUA
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|   | 1095 | CCUCCUC CUGAUGAGGCCGAAAGGCCGAA AACCUGU |
|---|------|----------------------------------------|
|   | 1105 | GUGAUGA CUGAUGAGGCCGAAAGGCCGAA AUCCUCC |
|   | 1107 | UGGUGAU CUGAUGAGGCCGAAAGGCCGAA AUAUCCU |
|   | 1110 | CGGUGGU CUGAUGAGGCCGAAAGGCCGAA AUGAUAU |
| 5 | 1119 | AGGCCUG CUGAUGAGGCCGAAAGGCCGAA ACGGUGG |
|   | 1119 | AGGCCUG CUGAUGAGGCCGAAAGGCCGAA ACGGUGG |
|   | 1119 | AGGCCUG CUGAUGAGGCCGAAAGGCCGAA ACGGUGG |
|   | 1127 | GGAGUAG CUGAUGAGGCCGAAAGGCCGAA AGGCCUG |
|   | 1127 | GGAGUAG CUGAUGAGGCCGAAAGGCCGAA AGGCCUG |
| 10 | 1130 | CUGGGAG CUGAUGAGGCCGAAAGGCCGAA AGGAGGC |
|   | 1133 | UUUCUGG CUGAUGAGGCCGAAAGGCCGAA AGUAGGA |
|   | 1146 | GUAGGAA CUGAUGAGGCCGAAAGGCCGAA AGCUUUU |
|   | 1146 | GUAGGAA CUGAUGAGGCCGAAAGGCCGAA AGCUUUU |
|   | 1148 | GUGUAGG CUGAUGAGGCCGAAAGGCCGAA AGAGCUU |
| 15 | 1149 | GGUGUAG CUGAUGAGGCCGAAAGGCCGAA AAGAGCU |
|   | 1152 | AGAGGUG CUGAUGAGGCCGAAAGGCCGAA AGGAAGA |
|   | 1158 | AAUCCAA CUGAUGAGGCCGAAAGGCCGAA AGGUGUA |
|   | 1160 | GAAAUCC CUGAUGAGGCCGAAAGGCCGAA AGAGGUG |
|   | 1165 | CACUGGA CUGAUGAGGCCGAAAGGCCGAA AUCCAAG |
| 20 | 1165 | CACUGGA CUGAUGAGGCCGAAAGGCCGAA AUCCAAG |
|   | 1166 | GCACUGG CUGAUGAGGCCGAAAGGCCGAA AAUCCAA |
|   | 1166 | GCACUGG CUGAUGAGGCCGAAAGGCCGAA AAUCCAA |
|   | 1245 | GGUUGGA CUGAUGAGGCCGAAAGGCCGAA ACAGCCU |
|   | 1246 | AGGUUGG CUGAUGAGGCCGAAAGGCCGAA AACAGCC |
| 25 | 1247 | CAGGUUG CUGAUGAGGCCGAAAGGCCGAA AAACAGC |
|   | 1247 | CAGGUUG CUGAUGAGGCCGAAAGGCCGAA AAACAGC |
|   | 1247 | CAGGUUG CUGAUGAGGCCGAAAGGCCGAA AAACAGC |
|   | 1268 | GGACUCG CUGAUGAGGCCGAAAGGCCGAA AGCGGCU |
|   | 1274 | CUGCAGG CUGAUGAGGCCGAAAGGCCGAA ACUCGGA |
| 30 | 1286 | GCGGAGA CUGAUGAGGCCGAAAGGCCGAA AGCUCUG |
|   | 1290 | GGGAGCG CUGAUGAGGCCGAAAGGCCGAA AGAGAGC |
|   | 1302 | CCGUGGC CUGAUGAGGCCGAAAGGCCGAA AUCAGGG |
|   | 1317 | CCUCCGG CUGAUGAGGCCGAAAGGCCGAA AUGCCCA |
|   | 1326 | GAGACAU CUGAUGAGGCCGAAAGGCCGAA ACCUCCG |
| 35 | 1331 | GAGCCGA CUGAUGAGGCCGAAAGGCCGAA ACAUGAC |
|   | 1333 | UCGAGCC CUGAUGAGGCCGAAAGGCCGAA AGACAUG |
|   | 1338 | CCACCUC CUGAUGAGGCCGAAAGGCCGAA AGCCGAG |

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        1349    GGCUGUG CUGAUGAGGCCGAAAGGCCGAA ACGCCAC
        1349    GGCUGUG CUGAUGAGGCCGAAAGGCCGAA ACGCCAC
        1350    GGGCUGU CUGAUGAGGCCGAAAGGCCGAA AACGCCA
        1350    GGGCUGU CUGAUGAGGCCGAAAGGCCGAA AACGCCA
   5    1359    UGUUCAU CUGAUGAGGCCGAAAGGCCGAA AGGGCUG
        1383    UUUCGAA CUGAUGAGGCCGAAAGGCCGAA AGGUCCA
        1385    GAUUUCG CUGAUGAGGCCGAAAGGCCGAA AGAGGUC
        1386    UGAUUUC CUGAUGAGGCCGAAAGGCCGAA AAGAGGU
        1392    GGUUGAU CUGAUGAGGCCGAAAGGCCGAA AUUUCGA
  10    1395    CGGGGUU CUGAUGAGGCCGAAAGGCCGAA AUGAUUU
        1407    GAGUGAU CUGAUGAGGCCGAAAGGCCGAA AUCUCGG
        1408    AGAGUGA CUGAUGAGGCCGAAAGGCCGAA AAUCUCG
        1408    AGAGUGA CUGAUGAGGCCGAAAGGCCGAA AAUCUCG
        1410    CGAGAGU CUGAUGAGGCCGAAAGGCCGAA AUAAUCU
  15    1414    CCAUCGA CUGAUGAGGCCGAAAGGCCGAA AGUGAUA
        1445    AAAACCG CUGAUGAGGCCGAAAGGCCGAA AGUCCAU
        1446    GAAAACC CUGAUGAGGCCGAAAGGCCGAA AAGUCCA
        1451    CUUGGGA CUGAUGAGGCCGAAAGGCCGAA AACCGAA
        1452    GCUUGGG CUGAUGAGGCCGAAAGGCCGAA AAACCGA
  20    1474    UGCAGGA CUGAUGAGGCCGAAAGGCCGAA AUCCACC
        1474    UGCAGGA CUGAUGAGGCCGAAAGGCCGAA AUCCACC
        1475    CUGCAGG CUGAUGAGGCCGAAAGGCCGAA AAUCCAC
        1476    UCUGCAG CUGAUGAGGCCGAAAGGCCGAA AAAUCCA
        1529    AUGGGCG CUGAUGAGGCCGAAAGGCCGAA AGACGUC
  25    1529    AUGGGCG CUGAUGAGGCCGAAAGGCCGAA AGACGUC
        1549    ACUCCCU CUGAUGAGGCCGAAAGGCCGAA ACCUCCA
        1580    GAGUUGG CUGAUGAGGCCGAAAGGCCGAA AGCCAUC
        1580    GAGUUGG CUGAUGAGGCCGAAAGGCCGAA AGCCAUC
        1587    ACAGAAG CUGAUGAGGCCGAAAGGCCGAA AGUUGGG
  30    1595    UCUUCAG CUGAUGAGGCCGAAAGGCCGAA ACAGAAG
        1595    UCUUCAG CUGAUGAGGCCGAAAGGCCGAA ACAGAAG
        1595    UCUUCAG CUGAUGAGGCCGAAAGGCCGAA ACAGAAG
        1624    CCCAGGG CUGAUGAGGCCGAAAGGCCGAA AUGCUGC
        1694    CAGCUGG CUGAUGAGGCCGAAAGGCCGAA AUCCGGA
  35    1787    CAGGCUA CUGAUGAGGCCGAAAGGCCGAA AGCCAGG
        1788    GCAGGCU CUGAUGAGGCCGAAAGGCCGAA AAGCCAG
        1816    CCAGAGA CUGAUGAGGCCGAAAGGCCGAA AUUUAGC
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1818    AGCCAGA CUGAUGAGGCCGAAAGGCCGAA AGAUUUA
1818    AGCCAGA CUGAUGAGGCCGAAAGGCCGAA AGAUUUA
1828    AGAGAGA CUGAUGAGGCCGAAAGGCCGAA ACAGCCA
1847    AUUCGUU CUGAUGAGGCCGAAAGGCCGAA ACUUGAG
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Table VI: Human CETP Hairpin Ribozyme and Substrate Sequence

| nt. Position | Ribozyme Sequence | Substrate |
|---|---|---|
| 27 | UUCCGGGC AGAA GGGUCU ACCAGAGAAACACGUUGUGGUACAUUACCUGGUA | AGACCCU GCU GCCCGGAA |
| 30 | CUCUUCCG AGAA GCAGGG ACCAGAGAAACACGUUGUGGUACAUUACCUGGUA | CCCUGCU GCC CGGAAGAG |
| 96 | GUGGCCCG AGAA GUUCAG ACCAGAGAAACACGUUGUGGUACAUUACCUGGUA | CUGAACG GCU CGGGCCAC |
| 119 | GGUUAUCA AGAA GUGGUG ACCAGAGAAACACGUUGUGGUACAUUACCUGGUA | CACCACU GCC UGAUAACC |
| 145 | AGGGUCAG AGAA GUGGCA ACCAGAGAAACACGUUGUGGUACAUUACCUGGUA | UGCCACA GUC CUGACCCU |
| 150 | GGGCCAGG AGAA GGACUG ACCAGAGAAACACGUUGUGGUACAUUACCUGGUA | CAGUCCU GAC CCUGGCCC |
| 162 | CAUUGCCC AGAA GGGCCA ACCAGAGAAACACGUUGUGGUACAUUACCUGGUA | UGGCCCU GCU GGGCAAUG |
| 182 | GCCUUUGG AGAA GGCAUG ACCAGAGAAACACGUUGUGGUACAUUACCUGGUA | CAUGCCU GCU CCAAAGGC |
| 235 | ACCAGGAG AGAA GGCUUG ACCAGAGAAACACGUUGUGGUACAUUACCUGGUA | CAAGCCU GCC CUCCUGGU |
| 276 | GGAAGGCG AGAA GGAUCA ACCAGAGAAACACGUUGUGGUACAUUACCUGGUA | UGAUCCA GAC CGCCUUCC |
| 280 | CGCUGGAA AGAA GUCUGG ACCAGAGAAACACGUUGUGGUACAUUACCUGGUA | CCAGACC GCC UUCCAGCG |
| 369 | AGUGGCUG AGAA GGAUGU ACCAGAGAAACACGUUGUGGUACAUUACCUGGUA | ACAUCCA GAU CAGCCACU |
| 490 | AGCCACCA AGAA GUGGUG ACCAGAGAAACACGUUGUGGUACAUUACCUGGUA | CACCACU GCC UGGUGGCU |
| 513 | AGUCAAUG AGAA GAUCAA ACCAGAGAAACACGUUGUGGUACAUUACCUGGUA | UUGAUCA GUC CAUUGACU |
| 552 | GUGUGUUG AGAA GGAGGU ACCAGAGAAACACGUUGUGGUACAUUACCUGGUA | ACCUCCA GAU CAACACAC |
| 564 | CACAGGUC AGAA GUGUGU ACCAGAGAAACACGUUGUGGUACAUUACCUGGUA | ACACACA GCU GACCUGUG |
| 567 | AGUCACAG AGAA GCUGUG ACCAGAGAAACACGUUGUGGUACAUUACCUGGUA | CACAGCU GAC CUGUGACU |

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591       GGGCAUCG AGAA GCACUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA GAGUGCG GAC CGAUGCCC
595       UCAGGGGC AGAA GUCCGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA GCGGACC GAU GCCCUGA
604       AGGUAGCA AGAA GGGGCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA UGCCCCU GAC UGCUACCU
615       UAUGGAAA AGAA GGUAGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA GCUACCU GUC UUUCCAUA
5         GAUGCAGG AGAA GCUUAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AUAAGCU CCU GCAUC
630
675       UUGUGAAC AGAA GCUUGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA UCAAGCA GCU GUUCACAA
678       AAUUGUG  AGAA GCUGCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AGCAGCA GUU CACAAAUU
726       CUUUGCAG AGAA GUCCCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AGGGACA GAU CUGCAAAG
10        UGGACAAA AGAA GCCAUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA CAUGGCC GAU UUUGUCCA
766
802       AUGUCUCC AGAA GAAAGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA CCUUUCA GAU GGAGACAU
853       AGGUAGGA AGAA GUGAUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA CAUCACA GCC UCCUACCU
942       AGUCCCCC AGAA GUGUGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA CCACACU GCU GGGGGACU
1025      GAGCAUGA AGAA GCCAUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA GAUGGCC GCC UCAUGCUC
1037      UCCCAUCA AGAA GAGCAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AUGCUCA GCC UGAUGGGA
15        CGUCUCCC AGAA GGCUGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA UCAGCCU GAU GGGAGACG
1041
1121      GCUGGGGA AGAA GCCGAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA GUCGGCG GCU UCCCCAGC
1147      AGGCAGUG AGAA GUGACU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA AGUCACC GUC CACUGCCU
1154      CAUCUUGA AGAA GUGGAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA GUCCACU GCC UCAAGAUG
20        UGUUGCUG AGAA GGGCGU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA ACGCCCA GAC CAGCAACA
1240
1291      GAGGCCUG AGAA GUAGUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA GACUACC GUC CAGGCCUC
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| | | Substrate |
|---|---|---|
| 1344 | UUGGUGUA AGAA GGAAAU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA | AUUCCA GAU UACACCAA |
| 1360 | AAGUUGGA AGAA GUCUUU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA | AAAGACU GUU UCCAACUU |
| 1382 | GGACUCGG AGAA GCUCUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA | GAGAGCA GCU CCGAGUCC |
| 1423 | AUGCCCAC AGAA GUGAUC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA | GAUCACC GCU GUGGGCAU |
| 1452 | CUACCUCG AGAA GAGACA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA | UGUCUCG GCU CGAGGUAG |
| 1471 | UUCAUGAG AGAA GUAAAC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA | GUUUACA GCC CUCAUGAA |
| 1545 | UCUGCAGC AGAA GGAAGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA | GCUUCCU GCU GCUGCAGA |
| 1548 | CCAUCUGC AGAA GCAGGA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA | UCCUGCU GCU GCAGAUGG |
| 1554 | CAAAGUCC AGAA GCAGCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA | UGCUGCA GAU GGACUUUG |
| 1581 | AAUCCACC AGAA GGUGCU ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA | AGCACCU GCU GGUGGAUU |
| 1669 | AGGGUUCC AGAA GUGAGC ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA | GCUCACA GCU GGAACCCU |

Table VII: Rabbit CETP Hairpin Ribozyme and Substrate Sequences

nt.

| Position | Ribozyme Sequence | Substrate |
|---|---|---|
| 57 | ACCAAGAG AGAA GGCUUG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA | CAAGCCC GCC CUCUUGGU |
| 98 | GGAAGGCC AGAA GGACCA ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA | UGGUCCA GAC GGCCUUCC |
| 102 | CGCUGGAA AGAA GUCUGG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA | CCAGACG GCC UUCCAGCG |
| 126 | CCGCUGAC AGAA GGAUAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA | CUAUCCG GAC GUCAGCGG |
| 160 | CUUGACCC AGAA GAGGAG ACCAGAGAAACACACGUUGUGGUACAUUACCUGGUA | CUCCUCG GCC GGGUCAAG |

| 191 | GGUGGCUG AGAA GGAGGU | ACCAGAGAAACACGUGUGGUACAUUACCUGGUA | ACCUCCA GAU CAGCCACC |
| 203 | UGGCGAUG AGAA GGUGGC | ACCAGAGAAACACGUGUGGUACAUUACCUGGUA | GCCACCU GUC CAUCGCCA |
| 335 | AGUCGACA AGAA GAUUGA | ACCAGAGAAACACGUUGUGGUACAUUACCUGGUA | UCAAUCA GUC UGUCGACU |
| 339 | UCGAAGUC AGAA GACUGA | ACCAGAGAAACACGUGUGGUACAUUACCUGGUA | UCAGUCU GUC GACUUCGA |
| 374 | CUGUGUUG AGAA GGAGGU | ACCAGAGAAACACGUGUGGUACAUUACCUGGUA | ACCUCCA GAU CAACACAG |
| 389 | CGUCGCAG AGAA GCUCUG | ACCAGAGAAACACGUGUGGUACAUUACCUGGUA | CAGAGCU GAC CUGCGACG |
| 426 | AGGUAGCA AGAA GGGGCA | ACCAGAGAAACACGUGUGGUACAUUACCUGGUA | UGCCCCC GAC UGCUACCU |
| 452 | GGUGCAGG AGAA GUUUAU | ACCAGAGAAACACGUGUGGUACAUUACCUGGUA | AUAAACU GCU CCUGCACC |
| 497 | UUGUGAAG AGAA GCUUGA | ACCAGAGAAACACGUGUGGUACAUUACCUGGUA | UCAAGCA GCU CUUCACAA |
| 533 | GCUUCAGA AGAA GCUUCA | ACCAGAGAAACACGUGUGGUACAUUACCUGGUA | UGAAGCU GAU UCUGAAGC |
| 588 | UGGACAAA AGAA GCCAUG | ACCAGAGAAACACGUGUGGUACAUUACCUGGUA | CAUGGCU GAC UUUGUCCA |
| 599 | CGGCCCUC AGAA GGACAA | ACCAGAGAAACACGUGUGGUACAUUACCUGGUA | UUGUCCA GAC GAGGGCCG |
| 624 | AUGUCUCC AGAA GAGAGG | ACCAGAGAAACACGUGUGGUACAUUACCUGGUA | CCUCUCA GAU GGAGACAU |
| 755 | GAAGACCG AGAA GGAAGG | ACCAGAGAAACACGUGUGGUACAUUACCUGGUA | CCUUCCC GCC CGGUCUUC |
| 760 | CCCCAGAA AGAA GGGCGG | ACCAGAGAAACACGUGUGGUACAUUACCUGGUA | CCGCCCG GUC UUCUGGGG |
| 801 | AGCACUUG AGAA GAGAAC | ACCAGAGAAACACGUGUGGUACAUUACCUGGUA | GUUCUCC GAU CAAGUGCU |
| 831 | UCCUGGAA AGAA GCCCUG | ACCAGAGAAACACGUGUGGUACAUUACCUGGUA | CAGGGCC GCC UUCCAGGA |
| 847 | GAGCACGA AGAA GCCCUC | ACCAGAGAAACACGUGUGGUACAUUACCUGGUA | GAGGGCC GUC UCGUGCUC |
| 859 | CCCUGUCA AGAA GAGCAC | ACCAGAGAAACACGUGUGGUACAUUACCUGGUA | GUGCUCA GCC UGACAGGG |
| 972 | AGGCAGUG AGAA GCUACC | ACCAGAGAAACACGUUGUGGUACAUUACCUGGUA | GGUAGCC GUC CACUGCCU |

| | | | | | |
|---|---|---|---|---|---|
| 979 | CACCUUAA | AGAA | GUGGAC | ACCAGAGAAACACACGUGUGUGGUACAUUACCUGGUA | GUCCACU GCC UUAAGGUG |
| 1035 | GUCACGGC | AGAA | GAAGAA | ACCAGAGAAACACACGUGUGUGGUACAUUACCUGGUA | UUCUUCC GUC GCCGUGAC |
| 1051 | GCGGGGGA | AGAA | GAACGU | ACCAGAGAAACACACGUGUGUGGUACAUUACCUGGUA | ACGUUCC GCU UCCCCGC |
| 1060 | GCCAUCUG | AGAA | GGGGAA | ACCAGAGAAACACACGUGUGUGGUACAUUACCUGGUA | UUCCCCC GCC CAGAUGGC |
| 1065 | UCUCGGCC | AGAA | GGGCGG | ACCAGAGAAACACACGUGUGUGGUACAUUACCUGGUA | CCGCCCA GAU GGCCGAGA |
| 1116 | GAGGCCUG | AGAA | GUGGUG | ACCAGAGAAACACACGUGUGUGGUACAUUACCUGGUA | CACCACC GUC CAGGCCUC |
| 1198 | AUUUGCUG | AGAA | GCCUGC | ACCAGAGAAACACACGUGUGUGGUACAUUACCUGGUA | GCAGGCA GCU CAGCAAAU |
| 1242 | AGGUUGGA | AGAA | GCCUUA | ACCAGAGAAACACACGUGUGUGGUACAUUACCUGGUA | UAAGGCU GUU UCCAACCU |
| 1253 | GGCUCUCA | AGAA | GGUUGG | ACCAGAGAAACACACGUGUGUGGUACAUUACCUGGUA | CCAACCU GAC UGAGAGCC |
| 1264 | GGACUCGG | AGAA | GCUCUC | ACCAGAGAAACACACGUGUGUGGUACAUUACCUGGUA | GAGAGCC GCU CCGAGUCC |
| 1291 | GAUCAGGG | AGAA | GAGAGA | ACCAGAGAAACACACGUGUGUGGUACAUUACCUGGUA | UCUCUCC GCU CCCUGAUC |
| 1298 | CCGUGGCG | AGAA | GGGAGC | ACCAGAGAAACACACGUGUGUGGUACAUUACCUGGUA | GCUCCCU GAU CGCCACGG |
| 1334 | CCACCUCG | AGAA | GAGACA | ACCAGAGAAACACACGUGUGUGGUACAUUACCUGGUA | UGUCUCG GCU CGAGGUGG |
| 1353 | UUCAUGAG | AGAA | GUGAAC | ACCAGAGAAACACACGUGUGUGGUACAUUACCUGGUA | GUUCACA GCC CUCAUGAA |
| 1423 | CAGCAGCA | AGAA | GCCAUC | ACCAGAGAAACACACGUGUGUGGUACAUUACCUGGUA | GAUGGCU GCC UGCUGCUG |
| 1427 | UCUGCAGC | AGAA | GGCAGC | ACCAGAGAAACACACGUGUGUGGUACAUUACCUGGUA | GCUGCCU GCU GCUGCAGA |
| 1430 | CCAUCUGC | AGAA | GCAGGC | ACCAGAGAAACACACGUGUGUGGUACAUUACCUGGUA | GCCUGCU GCU GCAGAUGG |
| 1436 | CGAAGUCC | AGAA | GCAGCA | ACCAGAGAAACACACGUGUGUGGUACAUUACCUGGUA | UGCUGCA GAU GGACUUCG |
| 1447 | CUGGGGAA | AGAA | GAAGUC | ACCAGAGAAACACACGUGUGUGGUACAUUACCUGGUA | GACUUCG GUU UUCCCAAG |
| 1463 | AAUCCACC | AGAA | GGUGCU | ACCAGAGAAACACACGUGUGUGGUACAUUACCUGGUA | AGCACCU GCU GGUGGAUU |

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10
15
20

1521   GCGGAGAC AGAA GCGUGU   ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA   ACACGCU GAC GUCUCCGC

1530   CCCCGAUG AGAA GAGACG   ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA   CGUCUCC GCC CAUCGGGG

1592   GUCUUCAG AGAA GAAGGA   ACCAGAGAAACACACGUGUGUGGUACAUUACCUGGUA   UCCUUCU GUC CUGAAGAC

1690   CAGCUGGG AGAA GGAGCA   ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA   UGCUCCG GAU CCCAGCUG

1697   UAGCAGGC AGAA GGGAUC   ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA   GAUCCCA GCU GCCUGCUA

1700   CGUUAGCA AGAA GCUGGG   ACCAGAGAAACACACGUGUGUGGUACAUUACCUGGUA   CCCAGCU GCC UGCUAACG

1727   ACCAGCAC AGAA GCUCCC   ACCAGAGAAACACACGUGUGUGGUACAUUACCUGGUA   GGGAGCA GCC GUGCUGGU

1763   GGACCUCA AGAA GGGUCU   ACCAGAGAAACACACGUGUGGUACAUUACCUGGUA   AGACCCA GAC UGAGGUCC

1793   ACUCACUG AGAA GGCUAA   ACCAGAGAAACACACGUGUGUGGUACAUUACCUGGUA   UUAGCCU GCC CAGUGAGU

1825   CAGAGAGA AGAA GCCAGA   ACCAGAGAAACACACGUGUGUGGUACAUUACCUGGUA   UCUGGCU GUC UCUCUCUG

1835   ACUUGAGA AGAA GAGAGA   ACCAGAGAAACACACGUGUGUGGUACAUUACCUGGUA   UCUCUCU GCC UCUCAAGU

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Claims

1.    Nucleic acid molecule which blocks synthesis and/or expression of mRNAs associated with initial development, progression or regression of vascular disease.

5        2.    The nucleic acid of claim 1 wherein said molecule is an enzymatic nucleic acid molecule.

3.    The nucleic acid molecule of claim 2 wherein said nucleic acid molecule cleaves mRNA produced from the gene encoding CETP.

10       4.    The nucleic acid molecule of claim 2, wherein the binding arms of said enzymatic nucleic acid molecule which contain sequences complementary to the sequences defined in any one of Tables II, IV, VI and VII.

5.    The enzymatic nucleic acid molecule of claims 2 15 or 3 or 4, wherein said nucleic acid molecule is in a hammerhead motif.

6.    The enzymatic nucleic acid molecule of claim 2 or 3 or 4, wherein said nucleic acid molecule is in a hairpin, hepatitis Delta virus, group I intron, VS nucleic 20 acid or RNaseP nucleic acid motif.

7.    The enzymatic nucleic acid molecule of any of claims 2 or 3 or 4, wherein said ribozyme comprises between 12 and 100 bases complementary to the RNA of said region.

25       8.    The enzymatic nucleic acid of claim 7, wherein said ribozyme comprises between 14 and 24 bases complementary to the RNA of said region.

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9. Enzymatic nucleic acid molecule consisting essentially of any sequence selected from the group of those shown in Tables III, V, VI and VII.

10. A mammalian cell including an enzymatic nucleic acid molecule of any of claims 1 or 2 or 3.

11. The cell of claim 10, wherein said cell is a human cell.

12. An expression vector comprising nucleic acid encoding the enzymatic nucleic acid molecule of any of claims 2 or 3 or 4, in a manner which allows expression and/or delivery of that enzymatic nucleic acid molecule within a mammalian cell.

13. A mammalian cell including an expression vector of claim 12.

14. The cell of claim 13, wherein said cell is a human cell.

15. A method for treatment of a patient having a condition associated with the level of CETP activity, wherein the said condition is selected from the group consisting of familial-hypercholestremia, atherosclerosis, peripheral vascular disease, dyslipidemia, hyperbetalipo-proteinemia, hypoalphalipoproteinemia, vascular complica-tions of diabetes, transplant, atherectomy and angio-plastic restenosis, wherein said patient is administered a therapeutically effective amount of an enzymatic nucleic acid molecule of claims 2 or 3 or 4.

16. A method for treatment of a condition related to the level of CETP activity by administering to a patient an expression vector of claim 12.

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17.   The method of claims 15 or 16, wherein said patient is a human.

FIG. I.

FIG. 2b.

FIG. 2a.

FIG. 2d.

FIG. 2c.

FIG. 3.

FIG. 4.

FIG. 5

I

630
C G̣ U
U

G—C
G—C
G—C
G—C
G—C
A C
A G
G A
620 •C—G• 640
G—C
U • G

Cleavage Site →

IV
680
A CUGÂAAUUGUCGUÂGCAGUU G
  | | | | | | | • | | | • | | • | A
U GACUUUAAC GUAUUGUCAUC
670                          700
          C   U G
          C   U   A
          U       U
          C—G
          C—G
          A—U  A
        660 •C—G
          U—A •720
          A C—G
          A A
          A  AA
          A  GCU GUG A CGGUAUUGGGCG U
  A GGGA  | | |   | | |   | • | | • | | • | A
5' gggaagcu • GUAGUAAGC  CCCGAACACGA—CAC  GUUAUGACUGA A
  • | | | | | | | • | | | | | | | | | | | — | | | • | • | |
3' UAAGAG CGUUCG—CCCGAACACGA—CAC  GUUAUGACUGA A
  650                    770              760
                                              G C
                                              A

V
690

III

730

740

750

VI

II
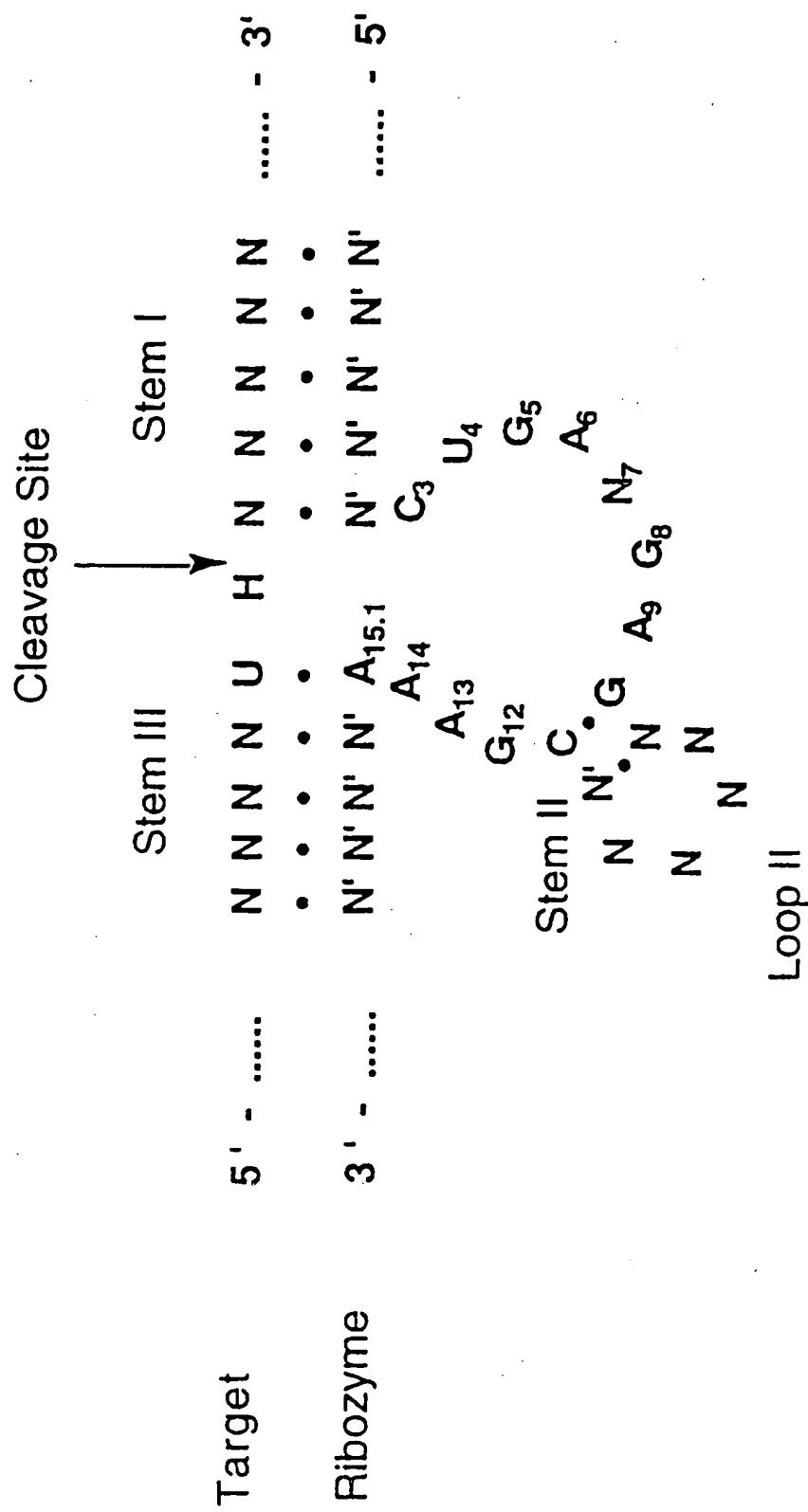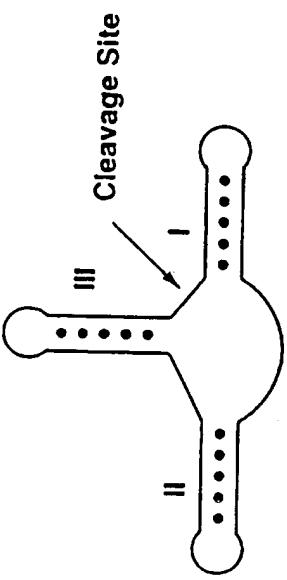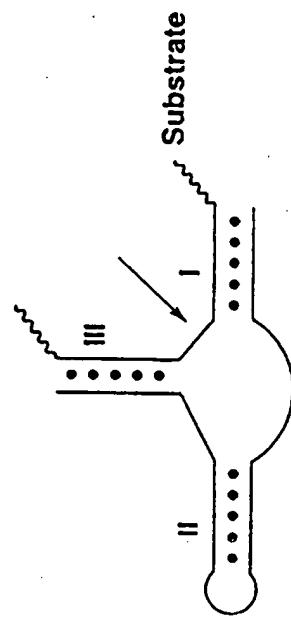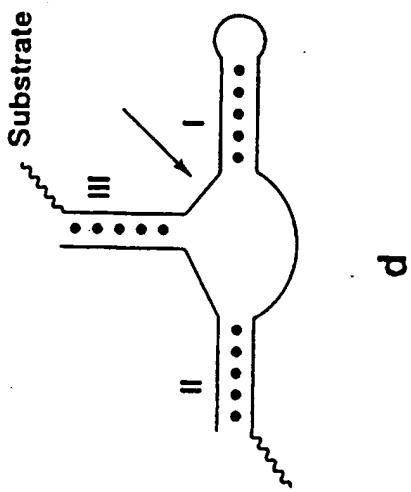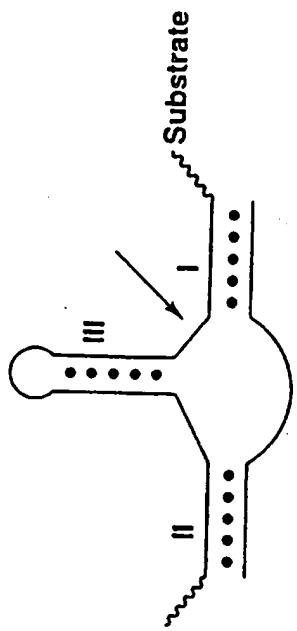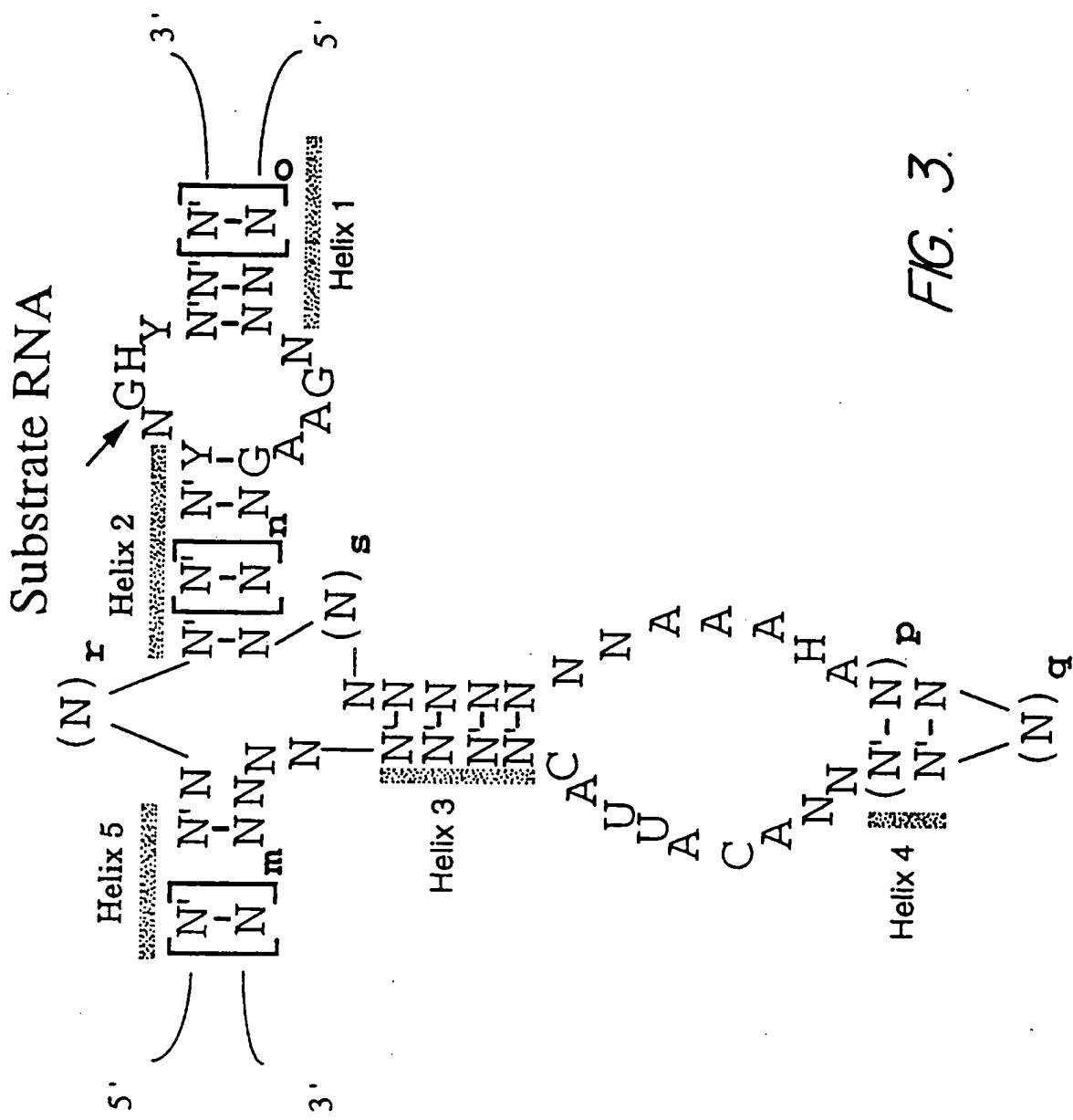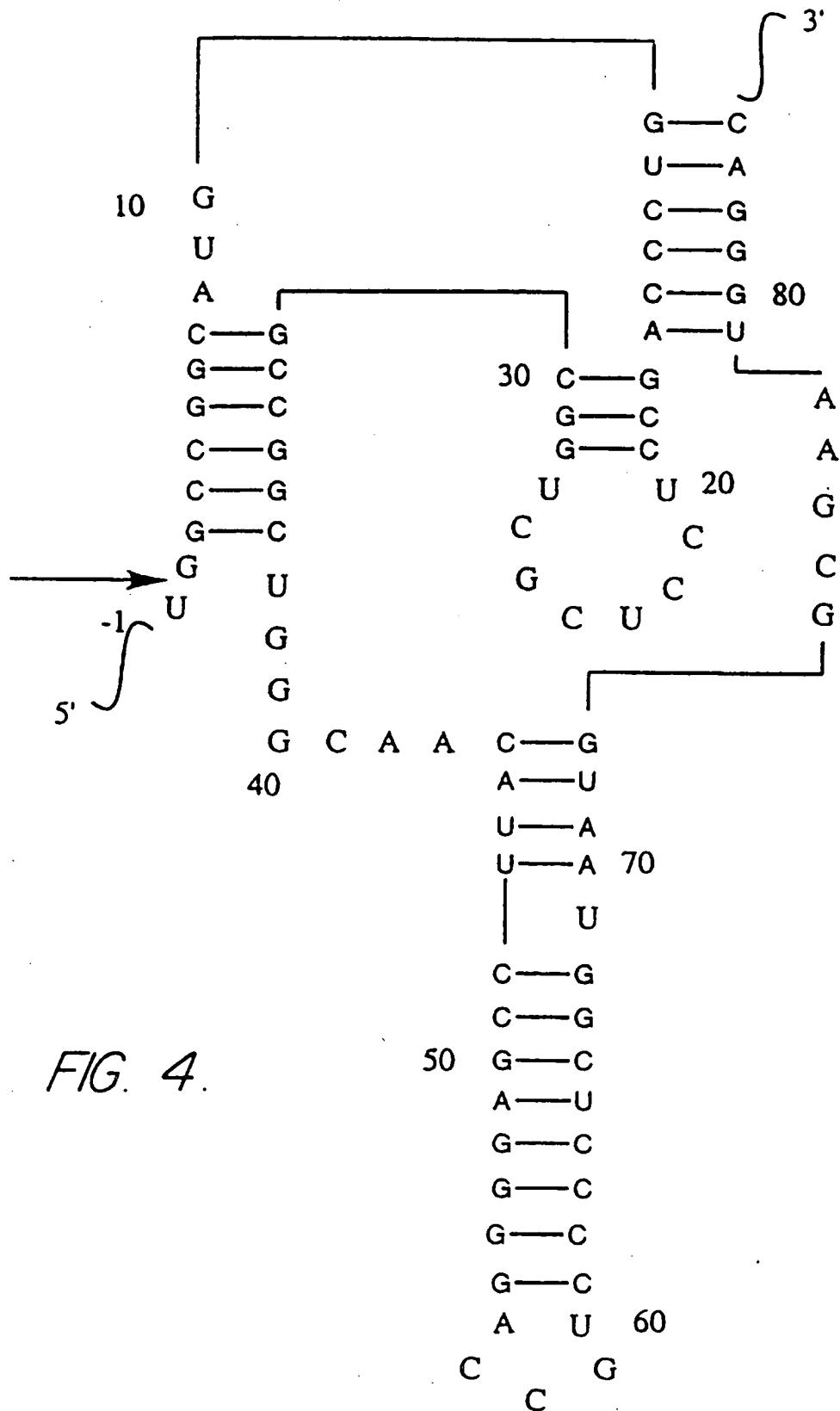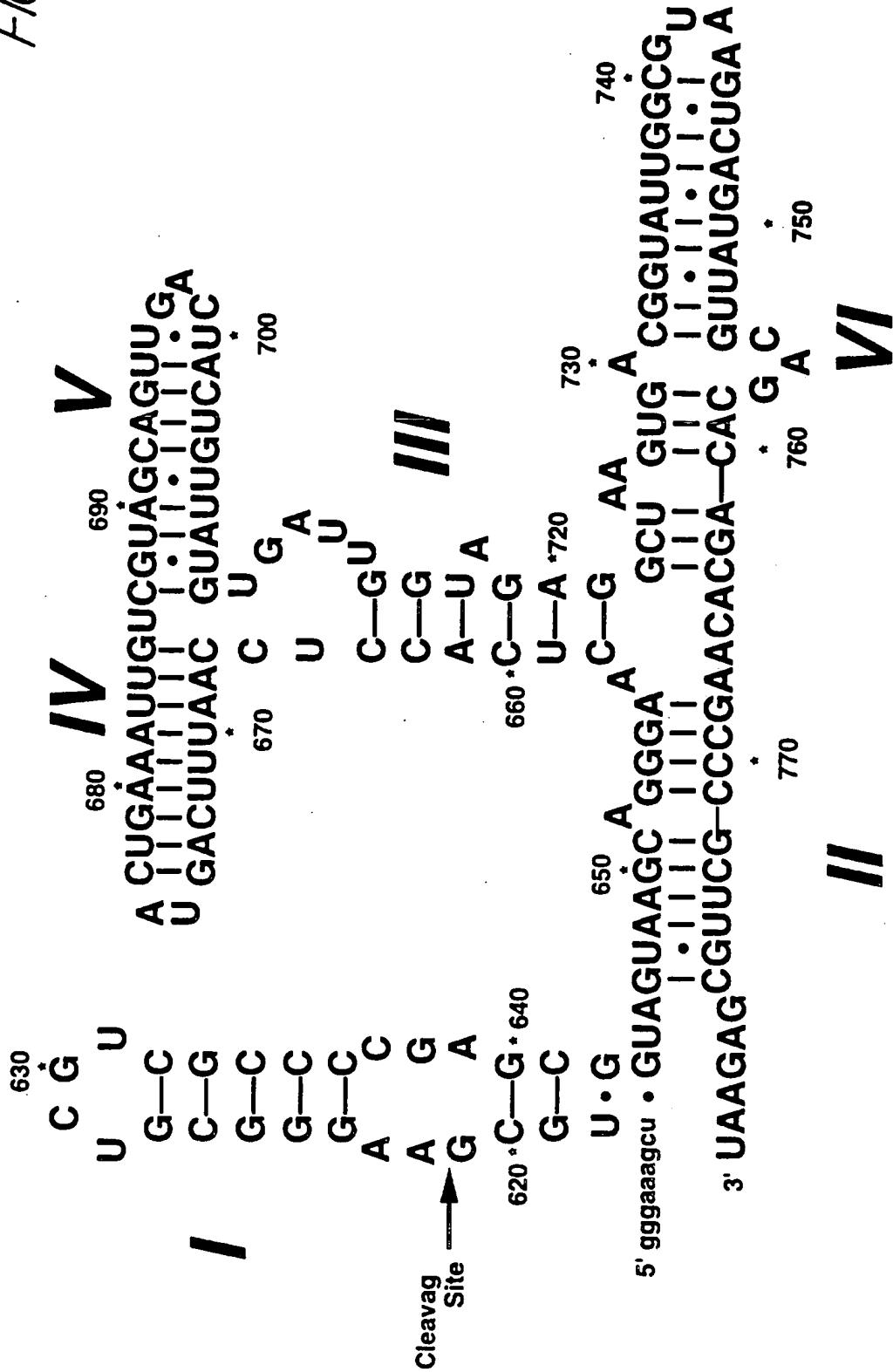
FIG. 6.
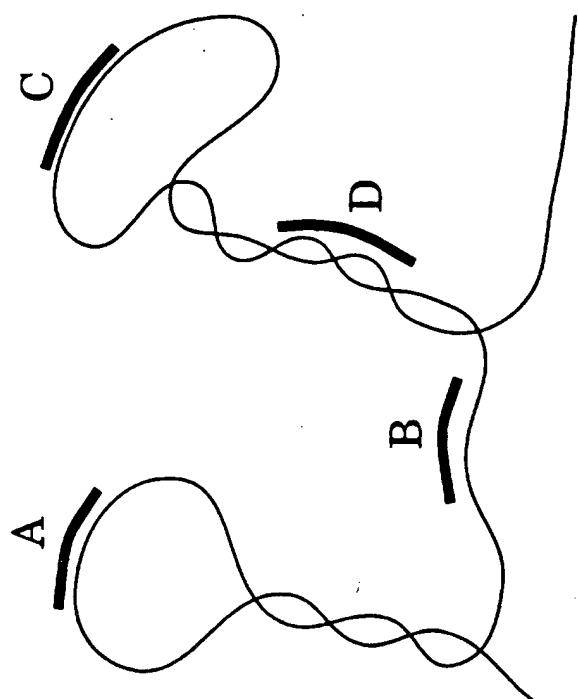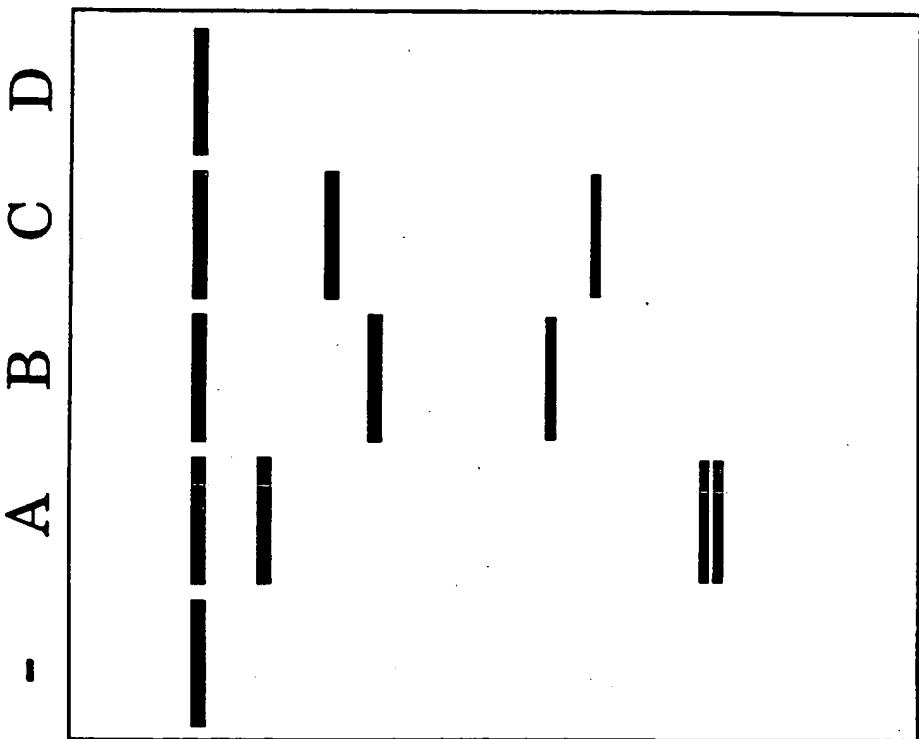
Body-labeled transcript
(not purified)
DNA oligo
(10 nM, 100 nM and 1000 nM)
• RNAse H
(0.08 -1.0 u/µl)
37°C, 10 min

# INTERNATIONAL SEARCH REPORT

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC 6  C12N15/52    C12N9/00    A61K31/70    C07H21/02    C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 6  C12N  C07H  A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO,A,94 02595 (RIBOZYME PHARMACEUTICALS INC) 3 February 1994 cited in the application see page 13, line 23 - line 35 | 1,2,5-8, 10-14 |
| Y | see page 21, line 9 - page 25 see claims | 1-17 |
| | --- | |
| Y | LIPIDS, vol. 29, December 1994, pages 811-818, XP000568834 BISGAIER, C. ET AL.: "Cholesteryl ester transfer protein inhibition by PD 140195" cited in the application see the whole document | 1-17 |
| | --- | |
| | -/-- | |

[X] Further documents are listed in the continuation of box C.    [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 6 May 1996 | 1 0. 05. 96 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016 | Andres, S |

Form PCT/ISA/210 (second sheet) (July 1992)

International Application No

PCT/US 95/16000

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | NATURE,<br>vol. 327, 18 June 1987, LONDON GB,<br>pages 632-634, XP002001819<br>DRAYNA, D. ET AL.: "Cloning and<br>sequencing of human cholesteryl ester<br>transfer protein cDNA"<br>cited in the application<br>--- | |
| P,X | JOURNAL OF BIOCHEMISTRY AND MOLECULAR<br>BIOLOGY 28 (3). 243-248,<br>31 May 1995, XP000569967<br>LEE, M. ET AL.: "Inhibitory effects of<br>antisense RNA on expression of cholesteryl<br>ester transfer protein in vaccinia virus<br>expression system."<br>see the whole document<br>----- | 1 |

1

| Box I | Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet) |

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 15-17
because they relate to subject matter not required to be searched by this Authority, namely:
**Remark: Although claims 15-17 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.**

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

| Box II | Observations where unity of invention is lacking (Continuation of item 2 of first sheet) |

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**        [ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

| Patent document cited in search report | Publication date | Patent family member(s) | | Publication date |
|---|---|---|---|---|
| WO-A-9402595 | 03-02-94 | AU-B- | 4769893 | 14-02-94 |
| | | CA-A- | 2140343 | 03-02-94 |
| | | EP-A- | 0654077 | 24-05-95 |
| | | JP-T- | 7509133 | 12-10-95 |